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(54) Title: TUMOR ASSOCIATED ANTIGEN 791Tgp72

(57) Abstract

Cancer vaccines comprising a polypeptide of the CD55 family, or fragments or derivatives of polypeptides of the CD55 family. A preferred polypeptide is the 791Tgp72 antigen. Also provided are isolated and purified 791Tgp72 antigens.

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Tumour Associated Antigen 791Tgp72

Field of the Invention

The present invention relates to tumour associated antigen 791Tgp72 and a method for its isolation, and to the use of 791Tgp72 and/or CD55 and related substances in methods of medical treatment, in particular as cancer vaccines.

Background of the Invention

A mouse monoclonal antibody 791T/36 was raised against the osteosarcoma cell line 791T (Embleton et al, 1981). A cell line expressing this antibody is deposited with the ATCC under accession number HB9173. Immunoprecipitation studies showed that 791T/36 recognised a membrane glycoprotein of molecular weight 72,000 (Price et al, 1984). A similar antigen can also be precipitated from activated human T lymphocytes. Extensive studies have shown that 791T/36 binds to the majority of osteosarcomas and also to colorectal, gastric and ovarian tumours (Durrant et al, 1986; Durrant et al, 1989; Durrant et al, 1989). The tumour specificity of 791Tgp72 was also emphasised by extensive clinical imaging studies with radiolabelled 791T/36 in the detection of primary and metastatic colorectal cancer, osteosarcoma, breast and ovarian cancer. The antibody was also liked to ricin A chain and showed good killing of tumour cells expressing the 791Tgp72 antigen. A phase I clinical study showed that the dose limiting toxicity was due to vascular leak syndrome and neurological toxicity of the ricin and was unrelated to antibody binding.

During the course of the clinical imaging and toxin targeting studies with 791T/36, it became clear that a

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limitation was the induction of human anti-mouse antibody
responses (HAMA) (Durrant et al, 1989) which could limit
the effectiveness of subsequent therapy with this
monoclonal antibody. A large component of this HAMA

5   response was directed at the idiotype of 791T/36. Most
patients made a very strong anti-idiotypic response
suggesting that a pre-existing helper T-cell response to
tumour expressed 791Tgp72 antigen allowed preferential
help for an anti-idiotypic response. Indeed a patient

10   who had already survived 3 years with metastatic colon
cancer received radiolabelled 791T/36 for tumour imaging.
He made a very strong idiotypic response which resulted
in anaphylactic shock suggesting that the pre-existing
helper response to the 791Tgp72 may have been stabilising

15   his disease and had been boosted with the injection of
791T/36. He recovered and lived a further 4 years
finally succumbing to bone metastases. A human
monoclonal anti-idiotypic antibody which bound to the
antigen combining site of 791T/36 was produced from this

20   patient (Austin et al, 1989 and WO90/04415). Similarly
immunisation of mice with 791T/36 linked to ricin induced
a strong anti-idiotypic response and a mouse monoclonal
anti-idiotypic antibody to 791T/36 was produced.

Clinical and laboratory studies with the human anti-

25   idiotypic antibody have shown that it is an excellent
immunogen for stimulating anti-tumour T-cell mediated
immunity. 105AD7 can prime delayed hypersensitivity
responses in rats and mice to human tumour cells
expressing 791Tgp72 antigen. No toxicity related to

30   anti-idiotypic immunisation has been observed in any of
the 164 patients entered into phase I/II clinical trials
with 105AD7. Patients in the phase I study showed T-cell
proliferation responses to both the 105AD7 immunogen and

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also to the target antigen 791Tgp72 which correlated with
survival. The lack of toxicity and excellent immune
responses has enabled us to undertake a trial in primary
colorectal cancer patients where evidence of autologous

5   anti-tumour cytotoxicity was observed in patients
immunised with 105AD7 prior to surgery. Single CTL
epitope vaccines may not be very effective as some tumour
cells lack the target antigen. This is less of a problem
when stimulating helper T-cell responses due to different

10   effector mechanisms. Antigen stimulation and homing
occur by a similar mechanism to CTL. However, once at
the tumour site, helper T-cells release cytokines which
initiate a cascade of inflammatory events resulting in
recruitment of effector cells which can kill tumour cells

15   independent of their antigen status. This kind of
infiltration profile has been seen in the tumours of
patients following 105AD7 immunisation. CD4 and CD8 T-
cells and natural killer cells were elevated in immunised
patients compared to unimmunised.. Furthermore, immunised

20   patients had enhanced natural killer cell activity, which
is of great significance as colorectal tumours often lose
expression of MHC molecules resulting in susceptibility
to NK killing.

25   Summary of the Invention
        Previous attempts to purify and identify the
791Tgp72 antigen using both immunoprecipitation and
affinity chromatography failed due to poor yields and the
conformational dependence of 791T/36 for antigen binding.

30   A modified method of affinity purification of 791Tgp72
has now been developed which has led to the isolation of
this antigen for the first time. Biotinylation of cell
membranes has allowed us to optimise the purification

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protocol, enabling efficient tracing of purified
fractions. The use of the mild detergent octyl-glucoside
and the introduction of an ultracentrifugation step has
enhanced the purification 50-100 fold. The affinity
chromatography has significantly been improved by
covalently coupling the capturing antibody (791T/36) to
protein-A sepharose. We have purified over 100 μg of the
antigen and N-terminal sequencing has identified the
molecule as being a member of the CD55/DAF family.

Further sequencing has revealed that the coding
region of 791Tgp72 cDNA is the same as that for a known
CD55 protein (herein known as "CD55"). Hence the amino
acid sequences of 791Tgp72 and CD55 are also identical.
There are, however, differences between the 791Tgp72 and
CD55 proteins, for example in the glycosylation pattern
of the molecules.

Accordingly, in a first aspect, the present
invention provides isolated and purified 791Tgp72
antigen.

In a further aspect, the present invention provides
isolated and purified 791Tgp72 antigen as obtainable by:

(a)   solubilising 791T cells in lysis buffer
including 1% octyl-B-glucoside, pH 8.5 for 1 hour at 4°C;

(b)   centrifuging the lysate at 13000 rpm x 10 min
following 100,000 g x 30 min;

(c)   adding the cleared lysate to Protein A
sepharose coupled to 791T/36 affinity column;

(d)   cycling the supernatant over the column at 0.3-
0.4 ml/min;

(e)   washing the column with 20ml 20 mM Tris-HCl pH
8.0 containing 0.3 M NaCl and 0.1% NP-40; and,

(f)   eluting 791Tgp72 from the column in 5 column
volumes of diethylamine pH 11.5 containing 0.5% NP-40 and

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neutralising the eluate with 1M Tris.

In a further aspect, the present invention provides a pharmaceutical composition comprising 791Tgp72 in combination with a pharmaceutically acceptable carrier.

5   In a further aspect, the present invention provides 791Tgp72 for use in a method of medical treatment.

In a further aspect, the present invention provides a method for isolating 791Tgp72 antigen from cells expressing 791Tgp72, the method including the steps of:

10   solubilising the cells with lysis buffer including octyl-glucoside; and

treating the lysate using ultracentrifugation.

The inventors found that these steps surprisingly helped to enhance the purification of the antigen 50-100

15   fold.

The isolation and characterisation of 791Tgp72 carried out for the first time here identified this antigen as a member of the CD55 or decay accelerating factor (DAF) family. Thus, the use of 791Tgp72 as a

20   cancer vaccine proposed herein can be extended to other CD55 polypeptides, a variety of forms of which have been isolated in the prior art, and to fragments and derivatives of these molecules. Likewise, the use of nucleic acid sequences encoding 791Tgp72 or its fragments

25   and derivatives can be extended to nucleic acid sequences encoding other CD55 family members, and their fragments and derivatives.

Accordingly, in a further aspect, the present invention provides a cancer vaccine comprising 791Tgp72

30   antigen or a polypeptide of the CD55 family, or a fragment or derivative of T791Tgp72 or of a polypeptide of the CD55 family, wherein the vaccine is capable of inducing an immune response in a patient. The response

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may be one or more of a T-helper cell response, a
cytotoxic T-cell response, an NK cell response and/or an
immune response.

In a further aspect, the present invention provides
a cancer vaccine comprising nucleic acid encoding
791Tgp72 and/or a polypeptide of the CD55 family, or a
fragment or derivative of T791Tgp72 or of a polypeptide
of the CD55 family, wherein the vaccine is capable of
inducing an immune response in a patient. Again, the
response may be one or more of a T-helper cell response,
a cytotoxic T-cell response, an NK cell response and/or
an immune response.

In a further aspect, the present invention provides
the use of 791Tgp72 antigen or a polypeptide of the CD55
family, or a fragment or derivative of T791Tgp72 or of a
polypeptide of the CD55 family, in the preparation of a
medicament for the treatment of cancer.

In a further aspect, the present invention provides
the use of nucleic acid encoding 791Tgp72 antigen or a
polypeptide of the CD55 family, or a fragment or
derivative of T791Tgp72 or of a polypeptide of the CD55
family, in the preparation of a medicament for the
treatment of cancer.

In a further aspect, the present invention provides
a method of treating a patient having cancer, the method
comprising administering to the patient a therapeutically
effective amount of one of the above cancer vaccines.

The use of 791Tgp72 to stimulate a T-cell response
to cancer cells bearing 791Tgp72 is unexpected, as it is
thought that the cancer cells have evolved to express
these antigens to protect them from complement mediated
attack. Thus, it is surprising that this defence
mechanism of the tumour cells provides a way of

selectively targeting a T-cell response to cancer cells
expressing high levels of these antigens. By way of
example, the vaccine can be used to treat colorectal
cancer, osteosarcoma, breast and ovarian cancer, all of
5   which are associated with 791Tgp72 overexpression.

While 791Tgp72 antigen and CD55 are known to be
over-expressed on a wide range of solid tumours, they are
also expressed on normal red blood cells, leukocytes,
endothelial cells and surface epithelial cells. However,
10   the T-cell response induced by employing a vaccine based
on these polypeptides should be capable of discriminating
between the low level of expression on normal cells and
the high levels on tumour cells. This is based on the
observation that the binding of 791T/36 to tumour cells
15   shows higher affinity than binding to red blood cells
from experiments in which the passage of red blood cells
through tumours resulted in transfer of the monoclonal
antibody to the tumour cells. Thus, this suggests that
the T-cell response will be targeted to tumours and
20   immune clearance is avoided.

Clinical studies with the human monoclonal antibody
105AD7 which mimics the colorectal tumour associated
antigen 791Tgp72 have shown that immunised patients show
a range of anti-tumour T-cell responses as exemplified by
25   antigen specific proliferation responses, enhanced IL-2
production, induction of CD45RO cells, infiltration of
CD4, CD8 and CD56 cells within the tumours of immunised
patients, enhanced natural killer activity and autologous
tumour killing which was unrelated to NK killing. As the
30   105AD7 antibody vaccine has now been given to 164
patients with no associated toxicity, vaccines based on
791Tgp72 or CD55 may share this property.

Results below supporting the use of polypeptides of

the CD55 family in an analogous manner to 791Tgp72
include:

(a) Sequence identity of 791Tgp72 and CD55.

(b) Monoclonal antibodies specific to CD55 bind to
purified 791Tgp72 antigen.

(c) 791T/36 binds to cells transfected with CD55.

(d) 791T/36 binds to cells transfected with
CD55/C46 chimeric constructs which contain CD55 SUSHI
domain 2.

(e) 791T/36 and monoclonal antibodies specific to
CD55 immunoprecipitate two proteins of 72 and 66 kDa from
the 791T osteosarcoma cell line. However, the yield of
the dimer is far greater with 791T/36 than with the anti-
CD55 monoclonal antibodies.

(f) 791T/36 and monoclonal antibodies specific to
CD55 immunoprecipitate a single band of 72kDa from normal
red blood cells.

(g) 791T/36 recognises an epitope on 791Tgp72 as
expressed by osteosarcoma cells, but binds weakly to red
blood cells. In contrast, BRIC 216 (Blood Group
Reference Laboratory, Bristol, UK), a monoclonal antibody
which recognises CD55 as expressed by red blood cell,
binds less well to osteosarcoma tumour cell lines as
compared to 791T/36.

(h) 791Tgp72 is a GPI linked protein which is
released by phospholipase C treatment.

(i) Radiolabelled 791T/36 localised within the
ovarian and colorectal tumours and showed no detectable
binding to red or white blood cells.

(j) 105AD7, an anti-idiotypic antibody which mimics
791Tgp72, has amino acid homology with the SCR2 domain
(also known as SUSHI domain 2) of CD55.

(k) 730, an anti-idiotypic antibody which mimics

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791Tgp72, has amino acid homology with the SCR2 domain of
CD55.

(1) Ab3 responses induced by either the human or
the mouse anti-idiotype may bind to CD55 on activated T-
5   cells and enhance proliferation.

Furthermore, analysis of the amino acid sequence of
CD55/791Tgp72 indicates that it may contain other T cell
epitopes, which are distinct from the epitopes mimicked
by 105AD7 and 730 anti-idiotypic antibodies. This
10   suggests that vaccines comprising these other epitopes
may induce immune responses in a broader range of
patients than vaccines prepared from the anti-idiotype
antibodies.

The present invention will now be described by way
15   of example and not limitation, with reference to the
accompanying figures.

Brief Description of the Figures
Figure 1 shows the detection of biotinylated
20   proteins following SDS-PAGE and Western blotting. 791T
cells were biotinylated and anti-DAF antibody (791T/36)
or control antibody (1143/B7) was added either before or
after solubilisation with 1% NP-40. The effect of
crosslinking reagent (DTSSP) was assessed in the
25   precipitation. X = Solubilisation after monoclonal
antibody incubation with cells for 1 hour. O =
Solubilisation of cells prior to addition of antibody.

Figure 2 shows SDS-PAGE analysis of 791T/gp72
immunoprecipitates from cell surface biotinylated 791T
30   cells. The samples were detected by the ECL reagent on
Western blotted gels. The gel represents the effects of
varying detergents and centrifugation protocols on sample
purification.

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Figure 3 shows SDS-PAGE and silver staining of samples during protein purification. Lane 1, protein marker; (2) cell lysate; (3) unbound sample after passing the column; (4-7) samples from four consecutive 5 ml

5   column washes; (8) concentrated washings; (9) samples of column eluate; and (10) concentrated column eluate. Each of the samples run on the gel was 25 μl volume. The washing and elution volumes were 5 ml.

Figure 4 shows analysis of affinity purified

10   791T/gp72 by 7% SDS-PAGE and detected by silver staining. Lanes 1-5, 25 μl samples from consecutive 1.2 ml diethylamine eluates from the Protein-A affinity column. Lanes 6-10, varying concentrations of purified BSA.

Figure 5 shows immunoprecipitation of cell surface

15   biotinylated 791T cells by antibodies to DAF (110,BRIC 216) anti 791T/gp72 (791T/36) and anti EGF receptor monoclonal antibody (340). Experiments were carried out with the same amount of antibody, analysed by SDS-PAGE and western blotting and detecting using the ECL system.

20   Lane 1 represents purified 791T/gp72. Lanes 4-7 represent precipitation with monoclonal antibodies 110, BRIC 216, 791T/36 and 340 respectively. Significantly more antigen is precipitated by 791T/36 compared to the anti-DAF antibodies.

25   Figure 6 shows binding of anti-DAF antibodies (110,BRIC 216) and 791T/36 to affinity purified 791Tgp72 antigen and to PI-PLC released antigen from 791T cells.

Figure 7 shows sandwich ELISA to determine if 791T/36 and the anti-DAF antibodies were binding distinct

30   domains. Plates were coated with 791T/36, control antibody 708 (IgG2b) or anti-DAF antibodies; 220 (SUSHI domain 1), 110 (SUSHI domain 2), BRIC 216 (SUSHI domain 3). Binding of 791Tgp72 was detected with FITC-791T/36.

Figure 8 shows non-reducing SDS-PAGE of proteins from erythrocyte and 791T cell membranes. Gels were Western blotted, cut into lanes, probed with appropriate antibody and developed using the ECL system.

5   Figure 9 shows clustal alignment of CD55 and cloned products from 791T cells. 5/P5 represents 5 clones sequenced from the primer sequence P5. RC of B/C DAF represents 5 clones sequenced from the primers B DAF 3' and c DAF 3'. CDAF.seq is the full length sequence of
10   CD55 taken from GENBANK.

Figure 10 shows the full length amino acid sequence of CD55 and the cDNA sequence which encodes it.

Figure 11 shows the full length cDNA sequence of 791Tgp72 and the deduced amino acid sequence. 791Tgp72
15   has been found to have an identical amino acid sequence to CD55 and to be encoded by cDNA which is identical over the entire coding region to the cDNA encoding CD55, though differences exist in the 5' and 3' non-coding regions. These differences may be attributable to the
20   use of different primers.

Detailed Description
CD55, DAF and 791Tgp72 Polypeptides

"791Tgp72" refers to the tumour associated antigen
25   isolated in the work described herein from 791T cells that is bound by antibody 791T/36 (Embleton et al; 1981). This antigen is a member of the CD55 family, and has a high degree of amino acid homology with this known polypeptide. However, there are other differences
30   between 791Tgp72 and other CD55 polypeptides, for example in the glycosylation pattern of the molecules. Further, different RNAs encoding 791Tgp72 antigen have been observed in the work described below and these may encode polypeptides having variations in amino acid sequence as
35   compared to CD55.

"CD55" refers particularly to the polypeptide having the sequence shown in figure 10. CD55 is also known as

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decay accelerating factor (DAF) and a variety of
alternative forms of the polypeptide are known.

As used herein, "CD55 family" includes sequences
which share substantial homology with CD55, such as the
5   aforementioned alternative forms of the polypeptide (e.g.
the previously identified CD55-A, CD55-B and CD55-U2),
and which are capable of inducing in a patient an immune
response against CD55 and/or 791Tgp72 as expressed on
cancer cells. Preferably the degree of homology between
10   CD55 and another protein of the CD55 family will be at
least 60%, more preferably 70%, further preferably 80%,
even more preferably 90%, or most preferably 95%.

CD55 was first purified by Nicholson-Weller et al
from guinea pig and human erythrocytes (see Nicholson-
15   Weller et al, 1981, 1982). Purified CD55 is a single
chain glycoprotein with an Mr of 60,000 (guinea pig) or
70,000 (human) on SDS-PAGE. CD55 is initially
synthesised as a precursor of 46 kDa, which gives rise to
the mature CD55 on the cell surface with an MW of 70,000
20   to 80,000 due to heterogeneity in glycosylation. The
structure of CD55 has been elucidated by a combination of
biochemical studies and by the molecular cloning of cDNA.
The cDNA for human CD55 encodes a 34-amino acid signal
peptide followed by a 347-amino acid sequence of the
25   protein. The amino terminus of the protein consists of
four CCPR domains (also known as SUSHI or SCR domains).
CD55 is anchored through covalent attachment to a GPI
anchor.

As shown herein, antigen 791Tgp72 has an identical
30   amino acid sequence to that of CD55 as shown in Fig 10.
The results described below suggest that CD55 and
791Tgp72, and fragments and derivatives thereof, can be
used as cancer vaccines, to induce immune responses such

as anti-tumour T-cell responses as exemplified by antigen specific proliferation responses, T-helper cell responses, cytotoxic T-cell responses, enhanced IL-2 production, induction of CD45RO cells, infiltration of CD4, CD8 and CD56 cells within the tumours of immunised patients, enhanced natural killer activity and/or autologous tumour killing which was unrelated to NK killing. Further, the peptides may act to raise CTL antibodies that neutralise CD55 and allow complement mediated lysis to take place.

Accordingly, the invention further includes the use of "fragments" or "derivatives" of either 791Tgp72 or other polypeptides of the CD55 family, which are less than the full length polypeptides, but which are capable of inducing an anti-tumour immune (especially T-cell) response as assessed by one or more of the indicators above. A preferred group of fragments are those which include all or part of the SUSHI2 domain of CD55 that stretches between amino acids 97-159 of full length CD55.

A "fragment" of a 791Tgp72 or of a polypeptide of the CD55 family means a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids, more preferably, at least about 20 to 30 or more contiguous amino acids, and most preferably at least about 30 to 40 or more consecutive amino acids.

A "derivative" of 791Tgp72 or of a polypeptide of the CD55 family, or of a fragment of 791Tgp72 or CD55 family polypeptide, means a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the

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natural amino acid sequence may involve insertion,
addition, deletion and/or substitution of one or more
amino acids, while providing a peptide capable of
inducing an anti-tumour T-cell response.

Preferably such derivatives involve the insertion,
addition, deletion and/or substitution of 25 or fewer
amino acids, more preferably of 15 or fewer, even more
preferably of 10 or fewer, more preferably still of 5 or
fewer and most preferably of 1 or 2 amino acids only.

The invention also includes derivatives of the above
peptides, including the peptide linked to a coupling
partner, e.g. an effector molecule, a label, a drug, a
toxin and/or a carrier or transport molecule. Techniques
for coupling the peptides of the invention to both
peptidyl and non-peptidyl coupling partners are well
known in the art. In one embodiment, the carrier
molecule is a 16 amino acid peptide derived from the
homeodomain of *Antennapedia* (e.g. as sold under the name
"Penetratin"), which can be coupled to a peptide via a
terminal Cys residue. The "Penetratin" molecule and its
properties are described in WO 91/18981.

Peptides may be generated wholly or partly by
chemical synthesis. The compounds of the present
invention can be readily prepared according to well-
established, standard liquid or, preferably, solid-phase
peptide synthesis methods, general descriptions of which
are broadly available (see, for example, in J.M. Stewart
and J.D. Young, Solid Phase Peptide Synthesis, 2nd
edition, Pierce Chemical Company, Rockford, Illinois
(1984), in M. Bodanzsky and A. Bodanzsky, The Practice of
Peptide Synthesis, Springer Verlag, New York (1984); and
Applied Biosystems 430A Users Manual, ABI Inc., Foster
City, California), or they may be prepared in solution,

by the liquid phase method or by any combination of
solid-phase, liquid phase and solution chemistry, e.g. by
first completing the respective peptide portion and then,
if desired and appropriate, after removal of any

5   protecting groups being present, by introduction of the
residue X by reaction of the respective carbonic or
sulfonic acid or a reactive derivative thereof.

Another convenient way of producing a peptidyl
molecule according to the present invention (peptide or

10   polypeptide) is to express nucleic acid encoding it, by
use of nucleic acid in an expression system.

Accordingly the present invention also provides in
various aspects nucleic acid encoding the polypeptides
and peptides of the invention.

15   Generally, nucleic acid according to the present
invention is provided as an isolate, in isolated and/or
purified form, or free or substantially free of material
with which it is naturally associated, such as free or
substantially free of nucleic acid flanking the gene in

20   the human genome, except possibly one or more regulatory
sequence(s) for expression. Nucleic acid may be wholly
or partially synthetic and may include genomic DNA, cDNA
or RNA. Where nucleic acid according to the invention
includes RNA, reference to the sequence shown should be

25   construed as reference to the RNA equivalent, with U
substituted for T.

Nucleic acid sequences encoding a polypeptide or
peptide in accordance with the present invention can be
readily prepared by the skilled person using the

30   information and references contained herein and
techniques known in the art (for example, see Sambrook,
Fritsch and Maniatis, "Molecular Cloning, A Laboratory
Manual, Cold Spring Harbor Laboratory Press, 1989, and

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Ausubel et al, Short Protocols in Molecular Biology, John
Wiley and Sons, 1992), given the nucleic acid sequence
and clones available. These techniques include (i) the
use of the polymerase chain reaction (PCR) to amplify

5   samples of such nucleic acid, e.g. from genomic sources,
(ii) chemical synthesis, or (iii) preparing cDNA
sequences. DNA encoding 791Tgp72 or CD55 fragments may
be generated and used in any suitable way known to those
of skill in the art, including by taking encoding DNA,

10   identifying suitable restriction enzyme recognition sites
either side of the portion to be expressed, and cutting
out said portion from the DNA. The portion may then be
operably linked to a suitable promoter in a standard
commercially available expression system. Another

15   recombinant approach is to amplify the relevant portion
of the DNA with suitable PCR primers. Modifications to
the sequences can be made, e.g. using site directed
mutagenesis, to lead to the expression of modified
peptide or to take account of codon preference in the

20   host cells used to express the nucleic acid.

In order to obtain expression of the nucleic acid
sequences, the sequences can be incorporated in a vector
having one or more control sequences operably linked to
the nucleic acid to control its expression. The vectors

25   may include other sequences such as promoters or
enhancers to drive the expression of the inserted nucleic
acid, nucleic acid sequences so that the polypeptide or
peptide is produced as a fusion and/or nucleic acid
encoding secretion signals so that the polypeptide

30   produced in the host cell is secreted from the cell.
Polypeptide can then be obtained by transforming the
vectors into host cells in which the vector is
functional, culturing the host cells so that the

polypeptide is produced and recovering the polypeptide
from the host cells or the surrounding medium.
Prokaryotic and eukaryotic cells are used for this
purpose in the art, including strains of E. coli, yeast,

5   and eukaryotic cells such as COS or CHO cells.

Thus, the present invention also encompasses a
method of making a polypeptide or peptide (as disclosed),
the method including expression from nucleic acid
encoding the polypeptide or peptide (generally nucleic

10   acid according to the invention). This may conveniently
be achieved by growing a host cell in culture, containing
such a vector, under appropriate conditions which cause
or allow expression of the polypeptide. Polypeptides and
peptides may also be expressed in in vitro systems, such

15   as reticulocyte lysate.

Systems for cloning and expression of a polypeptide
in a variety of different host cells are well known.
Suitable host cells include bacteria, eukaryotic cells
such as mammalian and yeast, and baculovirus systems.

20   Mammalian cell lines available in the art for expression
of a heterologous polypeptide include Chinese hamster
ovary cells, HeLa cells, baby hamster kidney cells, COS
cells and many others. A common, preferred bacterial
host is E. coli.

25   Suitable vectors can be chosen or constructed,
containing appropriate regulatory sequences, including
promoter sequences, terminator fragments, polyadenylation
sequences, enhancer sequences, marker genes and other
sequences as appropriate. Vectors may be plasmids, viral

30   e.g. 'phage, or phagemid, as appropriate. For further
details see, for example, Molecular Cloning: a Laboratory
Manual: 2nd edition, Sambrook et al., 1989, Cold Spring
Harbor Laboratory Press. Many known techniques and

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protocols for manipulation of nucleic acid, for example
in preparation of nucleic acid constructs, mutagenesis,
sequencing, introduction of DNA into cells and gene
expression, and analysis of proteins, are described in
5   detail in Current Protocols in Molecular Biology, Ausubel
et al. eds., John Wiley & Sons, 1992.

Thus, a further aspect of the present invention
provides a host cell containing heterologous nucleic acid
as disclosed herein.

10   The nucleic acid of the invention may be integrated
into the genome (e.g. chromosome) of the host cell.
Integration may be promoted by inclusion of sequences
which promote recombination with the genome, in
accordance with standard techniques. The nucleic acid
15   may be on an extra-chromosomal vector within the cell, or
otherwise identifiably heterologous or foreign to the
cell.

A still further aspect provides a method which
includes introducing the nucleic acid into a host cell.
20   The introduction, which may (particularly for *in vitro*
introduction) be generally referred to without limitation
as "transformation", may employ any available technique.
For eukaryotic cells, suitable techniques may include
calcium phosphate transfection, DEAE-Dextran,
25   electroporation, liposome-mediated transfection and
transduction using retrovirus or other virus, e.g.
vaccinia or, for insect cells, baculovirus. For
bacterial cells, suitable techniques may include calcium
chloride transformation, electroporation and transfection
30   using bacteriophage. As an alternative, direct injection
of the nucleic acid could be employed.

Marker genes such as antibiotic resistance or
sensitivity genes may be used in identifying clones

containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by
5   culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded polypeptide (or peptide) is produced. If the polypeptide
10 is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a polypeptide or peptide may be isolated and/or purified from the host cell and/or culture medium, as the case may
15 be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see
20 below).

Pharmaceutical Formulations
The polypeptides, derivatives and fragments of the invention can be formulated in pharmaceutical
25 compositions, and especially as vaccine compositions. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials
30 should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or

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subcutaneous, nasal, intramuscular, intraperitoneal
routes.   The formulation is preferably liquid, and is
ordinarily a physiologic salt solution containing non-
phosphate buffer at pH 6.8-7.6, or may be lyophilized
5   powder.

        The compositions comprising or for the delivery of
the 791Tgp72 and/or CD55 polypeptides are preferably
administered to an individual in a "prophylactically
effective amount" or a "therapeutically effective amount"
10   (as the case may be, although prophylaxis may be
considered therapy), this being sufficient to show
benefit to the individual.   The actual amount
administered, and rate and time-course of administration,
will depend on the nature and severity of what is being
15   treated.   Prescription of treatment, e.g. decisions on
dosage etc, is within the responsibility of general
practitioners and other medical doctors, and typically
takes account of the disorder to be treated, the
condition of the individual patient, the site of
20   delivery, the method of administration and other factors
known to practitioners.   The vaccines of the invention
are particularly relevant to the treatment of existing
cancer and in the prevention of the reoccurrence of
cancer after initial treatment or surgery.   Examples of
25   the techniques and protocols mentioned above can be found
in Remington's Pharmaceutical Sciences, 16th edition,
Oslo, A. (ed), 1980.

        791Tgp72 antigen and/or polypeptides of the CD55
family, and/or their fragments and/or derivatives are
30   prepared for administration by mixing them at the desired
degree of purity with adjuvants or physiologically
acceptable carriers, i.e. carriers which are non toxic to
recipients at the dosages and concentrations employed.

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Adjuvants and carriers are substances that in themselves share no immune epitopes with the target antigen, but which stimulate the immune response to the target antigen. Ordinarily, this will entail combining active ingredient with buffers, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose or dextrans, chelating agents such as EDTA, and other excipients. Freunds adjuvant (a mineral oil emulsion) has commonly been used for this purpose, as have a variety of toxic microbial substances such as mycobacterial extracts and cytokines such as tumour necrosis factor and interferon gamma. Other adjuvants for vaccination are disclosed in EP-A-0745388, WO97/01330 and EP-A-0781559. Carriers can also act as adjuvants, but are generally distinguished from adjuvants in that carriers comprise water insoluble macromolecular particulate structures which aggregate the antigen, typical carriers include aluminum hydroxide, latex particles, bentonite and liposomes.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Other cancer treatments include the 105AD7 antibody mentioned above, other chemotherapeutic agents, other radiotherapy techniques or other cancer vaccines known in the art. One particular application of the compositions of the invention are as an adjunct to surgery, i.e. to help to reduce the risk of cancer reoccurring after a tumour is removed.

It is envisioned that injections (intramuscular or subcutaneous) will be the primary route for therapeutic administration of the vaccines of this invention, intravenous delivery, or delivery through catheter or

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other surgical tubing is also used. Liquid formulations
may be utilized after reconstitution from power
formulations.

The polypeptide may also be administered via
microspheres, liposomes, other microparticulate delivery
systems or sustained release formulations placed in
certain tissues including blood. Suitable examples of
sustained release carriers include semipermeable polymer
matrices in the form of shaped articles, e.g.
suppositories, or microcapsules. Implantable or
microcapsular sustained release matrices include
polylactides (US Patent No:3,773,919, EP-A-0058481)
copolymers of L-glutamic acid and gamma ethyl-L-glutamate
(Sidman et al, Biopolymers 22(1): 547-556, 1985), poly(2-
hydroxyethyl-methacrylate) or ethylene vinyl acetate
(Langer et al, J. Biomed. Mater. Res. 15:167-277, 1981,
and Langer, Chem. Tech. 12:98-105, 1982). Liposomes
containing the polypeptides are prepared by well-known
methods: DE 3,218,121A; Epstein et al, PNAS USA,
82:3688-3692, 1985; Hwang et al, PNAS USA, 77:4030-4034,
1980; EP-A-0052522; E-A-0036676; EP-A-0088046; EP-A-
0143949; EP-A-0142541; JP-A-83-11808; US Patent Nos
4,485,045 and 4,544,545. Ordinarily, the liposomes are
of the small (about 200-800 Angstroms) unilamellar type
in which the lipid content is greater than about 30 mol.
% cholesterol, the selected proportion being adjusted for
the optimal rate of the polypeptide leakage.

The 791Tgp72 and/or peptides of the CD55 family may
be administered in a localised manner to a tumour site or
other desired site or may be delivered in a manner in
which it targets tumour or other cells.

Targeting therapies may be used to deliver the
active agent more specifically to certain types of cell,

by the use of targeting systems such as antibody or cell
specific ligands. Targeting may be desirable for a
variety of reasons, for example if the agent is
unacceptably toxic, or if it would otherwise require too

5   high a dosage, or if it would not otherwise be able to
enter the target cells.

        Instead of administering these agents directly, they
may be produced in the target cells by expression from an
encoding gene introduced into the cells, e.g. in a viral

10   vector (a variant of the VDEPT technique - see below).
The vector may targeted to the specific cells to be
treated, or it may contain regulatory elements which are
switched on more or less selectively by the target cells.

        The agent may be administered in a precursor form,

15   for conversion to the active form by an activating agent
produced in, or targeted to, the cells to be treated.
This type of approach is sometimes known as ADEPT or
VDEPT, the former involving targeting the activating
agent to the cells by conjugation to a cell-specific

20   antibody, while the latter involves producing the
activating agent, e.g. an enzyme, in a vector by
expression from encoding DNA in a viral vector (see for
example, EP-A-415731 and WO 90/07936).

        Vectors such as viral vectors have been used in the

25   prior art to introduce nucleic acid into a wide variety
of different target cells. Typically the vectors are
exposed to the target cells so that transfection can take
place in a sufficient proportion of the cells to provide
a useful therapeutic or prophylactic effect from the

30   expression of the desired polypeptide.

        A variety of vectors, both viral vectors and plasmid
vectors, are known in the art, see US Patent No.
5,252,479 and WO 93/07282. In particular, a number of

viruses have been used as gene transfer vectors,
including papovaviruses, such as SV40, vaccinia virus,
herpesviruses, including HSV and EBV, and retroviruses.
Many protocols in the prior art have used disabled murine
retroviruses.

As an alternative to the use of viral vectors other
known methods of introducing nucleic acid into cells
includes electroporation, calcium phosphate co-
precipitation, mechanical techniques such as
microinjection, transfer mediated by liposomes and direct
DNA uptake and receptor-mediated DNA transfer.

Receptor-mediated gene transfer, in which the
nucleic acid is linked to a protein ligand via
polylysine, with the ligand being specific for a receptor
present on the surface of the target cells, is an example
of a technique for specifically targeting nucleic acid to
particular cells.

The vaccination dose of the 791Tgp72 or CD55 family
polypeptide will be dependent upon the properties of the
vaccine employed, e.g. its binding activity and in vivo
plasma half-life, the concentration of the polypeptide in
the formulation, the administration route, the site and
rate of dosage, the clinical tolerance of the patient
involved, the pathological condition afflicting the
patient and the like, as is well within the skill of the
physician. For example, doses of 300 μg of polypeptide
per patient per administration are preferred, although
dosages may range from about 10 μg to 1 mg per dose.
Different dosages are utilized during a series of
sequential inoculations; the practitioner may administer
an initial inoculation and then boost with relatively
smaller doses of vaccine.

The vaccine compositions of the invention can be

administered in a variety of ways and to different classes of recipients. Examples of types of cancer that can be treated with the vaccine include colorectal cancer, osteosarcoma, breast and ovarian cancers.

This invention is also directed to optimized immunization schedules for enhancing a protective immune response against cancer. By way of example, at least three separate inoculations with 791Tgp72 and/or CD55 family polypeptides be administered, with a second inoculation being administered more than two, preferably three to eight, and more preferably approximately four weeks following the first inoculation. It is preferred that a third inoculation be administered several months later than the second "boost" inoculation, preferably at least more than five months following the first inoculation, more preferably six months to two years following the first inoculation, and even more preferably eight months to one year following the first inoculation. Periodic inoculations beyond the third are also desirable to enhance the patient's "immune memory". See Anderson et al, J Infectious Diseases 160 (6):960-969, Dec.1989 and the references therein. Generally, infrequent immunizations with polypeptides spaced at relatively long intervals is more preferred than frequent immunizations in eliciting maximum antibody responses, and in eliciting a protective effect.

The above discussion, insofar as it relates to vaccine compositions and to the production of nucleic acid compositions, is generally applicable also to the nucleic acid vaccines of the present invention, in accordance with the following comments which relate specifically to such vaccines.

Nucleic acid immunisation involves the use of a

nucleic acid, usually DNA, vector encoding a gene of
interest. A preferred vector is pcDNA3 (Invitrogen,
Groningen, Netherlands). A DNA sequence encoding the
gene of interest is typically placed under the control of

5 a eukaryotic promoter that allows for expression in the
target mammalian cells. By inclusion of various known
sequence tags the encoded gene product may be directed to
various compartments within the cell. This may be used
to influence the direction of the developing immune

10 response, for example favouring CTL or antibody
responses.

The vector is introduced into the mammalian body by
a number of possible routes. For example, injection of a
naked DNA vector into muscle or via an intradermal route

15 has been successful in establishing immune responses, a
typical protocol involving the intramuscular injection of
$50\mu g$ DNA into two muscles on three occasions. Other
possible routes include encapsulation of the nucleic acid
vector into particles that are taken up by antigen-

20 presenting cells. Poly(lactide-coglycolide) PLG
microparticles have been successfully used to raise
immune responses by feeding the particles to mice.

A major advantage of nucleic acid immunisation is
the prolonged production of immunogen from within the

25 cells of the immunised mammal, in a similar way to that
of viral infections. The vector nucleic acid has also
been shown to be a stimulator of innate immunity,
providing the right environment in which to establish an
efficient and sustained immune response.

30

Example 1
Identification of 791Tgp72 Antigen by Immunoprecipitation

To improve the yield of 791Tgp72 antigen, both

immunoprecipitation and affinity chromatography
biotinylation of cell membranes was used to optimise the
purification protocol, enabling efficient tracing of
purified fractions. Cell culture and surface

5   biotinylation were carried out as described in Altin et
al, 1994. The cell line 791T was cultured in RPMI 1640
medium supplemented with heat-inactivate 10% fetal calf
serum (FCS). The cells were harvested with trypsin/EDTA
and washed three times with ice-cold PBS-C/M before

10   reacting with 0.5 mg/ml sulfo-NHS-biotin (Pierce) for 30
minutes at 4°C. In some experiments, biotinylation was
carried out in the presence of the chemical cross-linking
agent 3,3-dithio-bis(sulfo-succinimidyl-propionate)
(DTSSP; Pierce) to covalently link associated molecules.

15   For these studies, cells were suspended in phosphate
buffered saline minus $CaCl_2/MgCl_2$ (PBS-C/M, pH 7.6),
biotinylated and then crosslinked for 1 hr at room
temperature with gentle mixing, following the
manufacturers recommendations (PIERCE). Initial

20   precipitations were carried out on biotinylated samples.
Antibody (791T/36) was added to either whole cells or
cell lysates. For these experiments 1143/B7 Mab was used
as the negative control antibody.

Cells ($2-5 \times 10^7$) were lysed for 2 hrs at 4°C, cell

25   lysates were cleared by centrifugation at 13000 rpm for
15 minutes. Immune complexes were then formed with
protein-A sepharose (Sigma) for 30 minutes at 4°C. This
basic protocol allowed us to vary the detergents and
their concentrations, washing conditions and incubation

30   times in order to optimise the purification protocol.
Detergents tested were 0.5, 1.0 and 1.5 %; Nonidet P-40,
Tween-20 and Octyl Glucoside. These were used in TNE
(20mM Tris, pH 8.0, 140 mM NaCl, 5mM EDTA). Washes were

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carried out with (20mM Tris pH 8.0, 100 mM NaCl, 1mM
EDTA, 0.1 mM PMSF + 0.25 % detergent). Washed protein-A
sepharose beads were boiled in sample buffer (+/-)
mercaptoethanol, reducing or non-reducing conditions.

5   The samples were analysed by SDS-PAGE, Western blotted
onto nitrocellulose membrane (Hybond™-C; Amersham), and
detection of biotinylated proteins were carried out as
described in Laemmli (1970), Stern (1993) and Dunbar
(1994).

10   After transferring, the membrane was briefly washed
with PBS and dried for 30 minutes at room temperature
before blocking with PBS containing 0.1% Tween-20 and 1%
BSA. The membrane was then washed twice for 5 minutes
with PBS containing 0.1% Tween-20 and then incubated with
15   horseradish peroxidase (HRPO)-streptavidin (1:1500;
GIBCOBRL) for 1 hour at room temperature. The membrane
was then washed three times (as above) and proteins were
detected using the enhanced chemiluminescence (ECL)
protein detection system (Amersham) by exposing the
20   chemiluminescent blot to X-OGRAPH film. The detection of
non-biotinylation proteins was carried out by silver
staining.

Figure 1 shows the results of immunoprecipitation by
mAb 791/36 from 791T cells. All cells were solublised in
25   TNEN buffer containing 1% NP-40. The binding reaction
was carried out before (Lanes 1-4) or after (Lanes 5-8)
cell solubilisation. Cross linking reagent (DTSSP) was
used in some of the reactions (Lanes 3, 4, 7 and 8) and
the precipitation was carried out using 79T/36 (odd lane
30   numbers) or 1143/B7 control antibody (even lane
numbers). It can be seen that crosslinking the antibody
to the cell surface (Lane 3) improved the amount of
purified antigen compared to cells solublised without

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crosslinking.   Comparable results were obtained with
cells solublised prior to antibody addition (lanes 5 and
7).

5   Example 2
        Checking the Conditions for Purification of the Antigen
               In order to optimise the conditions for the
        purification of large amounts of 791Tgp72 the conditions
        were varied as for purification of the biotinylated
10   protein.   Initially, CNBr-activated sepharose 4B was used
        to make an affinity column with 791T/36 Mab (see Hole et
        al, 1988; Hole et al, 1990; Goding, 1996), but this
        proved very inefficient.   A modification of this
        procedure using Protein A sepharose was introduced
15   (Scneider et al, 1982). 1-2 x 10^9 791T cells were
        solubilized in 100 ml of 1% octyl glucoside in Tris
        buffer (20 mM Tris-HCl pH 8.5, 150 mM NaCl, 25 mM
        benzamidine, 5 mM EGTA, 10 ug /ml leupeptin, 0.1 mM PMSF)
        for 1 hr at 4°C with continual mixing.   Unsolubilized
20   material was discarded after centrifugation at 13000 rpm
        for 10 minutes, this was followed by a 100,000 g
        centrifugation of the supernatant for 30 minutes.   The
        solubilized material was loaded on to the protein-A
        Sepharose-791T/36 crosslinked affinity column with a flow
25   rate of 0.3-0.4 ml/min.   The column was then washed with
        20 ml 50 mM Tris-HCl pH 8.0 containing 0.3 M NaCl with
        0.1% NP-40.   The 791Tgp72 antigen was eluted with 5
        column volume of 50 mM diethylamine pH 11.5 containing
        0.5% NP-40.   The sample was immediately neutralised by
30   adding 200 ul of 1 M Tris-HCl pH 8.0.   The original
        sample was recycled over the column another 2-3 times as
        above to recapture any unpurified antigen.   Fractions
        were assessed by SDS-PAGE and silver staining.

30

Figure 2 shows the effects of various conditions on purification efficiency. Lane 2-4 represent three cell lysates solubilized by different non-ionic detergents. Octyl-B-glucoside yielded more precipitates (lane 2) and

5   ultracentrifugation when used achieved significant improvements in reducing background protein contamination. Using the 791T36-protein-A sepharose column and similar conditions we were able to show significant improvements in yields of antigen (Figure 3,

10   lane 10). However, we also showed that antigen was also eluted by even the mildest washing conditions (Figure 3, lane 3-9)

Following analysis of the purification procedures, the final conditions were chosen for affinity

15   chromatography:

(1)   Lysis buffer with 1% octyl-B-glucoside, pH 8.5 for 1 hour at 4°C.

(2)   Lysate centrifugation: 13000 rpm x 10 min following 100,000 g x 30 min.

20   (3)   Addition of cleared lysate to Protein A sepharose coupled to 791T/36 affinity column.

(4)   Cycle the supernatant over the column at 0.3-0.4 ml/min.

(5)   Washing the column with 20ml 20 mM Tris-HCl pH 8.0

25   containing 0.3 M NaCl and 0.1% NP-40.

(6)   Sample was eluted in 5 column volumes of diethylamine pH 11.5 containing 0.5% NP-40 and neutralised with 1M Tris.

(7)   The sample solution was recycled for 3-4 times to

30   recover as much antigen as possible.

Example 3

Sequence Analysis

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To determine the N-terminal amino acid sequence, affinity purified 791Tgp72/66 was concentrated using vivaspin centrifugation columns. Approximately 10μg of protein was analysed by SDS-PAGE and Western blotted onto

5   PVDF membrane (PROBLOT, ABI) following the manufacturer's recommendations, with a modification by addition of 0.1% SDS. Following transfer for 1-2 hrs, the blot was stained with Coomassie blue for 30 seconds and rinsed in 10 % methanol 20% acetic acid. The stained 66 and 72 KDa

10   bands were excised from the blot and subjected to 16 rounds of automated protein sequencing on an ABI XXX sequencer.

Figure 4 shows the results of silver staining from the fractions of protein A column. The antigens of

15   791Tgp72 and p66 were eluted in 2-3 fractions.

N-terminal sequence analysis gave the following sequence "DCGLPPDVPNAQPALE" which showed 100% identity with the sequence of decay accelerating factor (DAF, CD55).

20

Example 4

Transfection of CD55 into CHO Cells

To check the recognition of CD55 by 791T/36 Mab, CHO cells were transfected with a CD55 cDNA clone. The clone

25   was obtained from Dr Dale Christiansen (Austin Research Institute, Victoria 3084, Australia). Cells transfected with the clone were assayed by FACS analysis for binding of anti-CD55 antibodies, 110 and BRIC 216 and also for binding of 791T/36. All the antibodies showe good

30   binding to the CHO cells transfected with CD55 but no binding to untransfected cells, see Table 1.

Example 5

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Flow Cytometry Anti-CD55 Binding Assay

To measure expression of 791Tgp72 and CD55 on tumour and primary cell lines, 2×10^5 of 791T, human umbilical vein endothelial cells (HUVEC) and erythrocytes were mixed with cold anti-CD55 110, BRIC 216, 220, 791T/36 and control mAb 708 (0.1 µg) separately at 37°C for 1 hr. Then rabbit anti-mouse FITC (1:100, DAKO, Denmark) was added to each tube and incubated for another 1 hr. Direct binding of 791T/36 FITC (0.1 µg) to 791T cells was measured after 1hr at 37°C. The cells were washed two times with RPMI 1640 medium, fixed and measured by flow cytofluorometry.

Table 2 present the results of antibodies binding to different cell lines. The data show that both anti-CD55 and 791T/36 mabs bind to red blood cells, HUVEC cells and to the osteosarcoma cell line 791T. The anti-CD55 antibody BRIC 216 bound most strongly to red blood cells and HUVEC cells whereas 791T/36 showed the strongest binding to 791T cells which was approximately 2 orders of magnitude higher than to the normal cells. These results suggest that 791Tgp72 is closely related to CD55 but that there are some differences. These differences could be differential glycosylation or post-translational modifications, e.g. point mutations.

Example 6

Immunoprecipitation with Various Anti-CD55 Antibodies

To confirm whether anti-CD55 monoclonal antibodies could precipitate an antigen from tumour cells, the same immunoprecipitation protocol as mentioned previously was used. 40 µg of anti-CD55 110, BRIC 216 and anti-791Tgp72, 91T/36 were used to precipitate the antigen from 2×10^7 791T cells respectively.

Both anti-CD55 monoclonal antibodies, 110, BRIC 216 and 791T36 immunoprecipitated an antigen of similar molecular weight although the yield was far greater with 791T/36 than the with the anti-CD55 antibodies (Figure 5). These results again suggest that a similar antigen is precipitated by both the anti-CD55 antibodies and 791T/36, but that the later Mab has either better access or a higher affinity for 791Tgp72.

Example 7

Phosphatidylinositol Phospholipase C (PI-PLC) Treatment

CD55 is a GPI linked protein. To confirm whether 791Tgp72 is also GPI linked, 791T cells were treated with Phosphatidylinositol phospholipase C (PI-PLC; Boehringer Mannheim, Germany), to release GPI linked antigens. Cells (5×10^5) were incubated with PI-PLC(1 U/ml) for 1 hour at 37°C. The cells were washed two times with PBS and the expression of CD55 and or 791Tgp72 was determined by indirect immunofluorescence binding with monoclonal antibodies and flow cytometric analysis.

As shown in Table 3, the binding of anti-CD55 monoclonal antibodies and 791T/36 decreased after incubation with PI-PLC for 1 hr, with a maximal decrease in surface expression of approximately 85-90%. Cells incubated in parallel without PI-PLC retained their surface expression of 791Tgp72 antigen. These results clearly show that 791Tgp72 is also GPI linked.

Example 8

Purified 791Tgp72 Antigen

To confirm that the anti-CD55 mabs can bind to 791Tgp72, purified antigen (50 ng) or antigen released form PI-PLC treated 791T cells was added separately to

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flexible microtest plates (Falcon, Becton Dickinson, CA, USA) and left at 37°C till dry. The plates were washed three times with phosphate buffered saline containing 0.05% Tween-20 (PBS-Tween) and blocked with BSA (1%) for

5   1 hr at room temperature. The plates were washed three times, then anti-CD55 antibodies (500 ng) in PBS were added. After 1hr at room temperature the plates were washed three times in PBS-Tween and conjugated rabbit anti-mouse horseradish peroxidase (HRPO) diluted 1:1000

10   was added for a further 1hr. Finally, after extensive washing, the plates were developed and read at 405 nm.

The binding of 791Tgp72 antigen to mAb 791T/36 and other anti-CD55 antibodies was shown by ELISA. The binding of both 791T/36 and anti-CD55 antibodies to

15   purified 791Tgp72 antigen was clearly seen. 791T/36 also showed significant binding to 791Tgp72 antigen released from PI-PLC treated 791T cells (Figure 6). The binding of the anti-CD55 mAbs to purified 791Tgp72 confirm that this antigen shares considerable homology with CD55.

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Example 9

Mapping of the 791T/36 Epitope

DAF (CD55) consists of 4 SUSHI domains, a C-terminal O-glycosylated tail and a GPI anchor. Purified 791Tgp72

25   was used in a sandwich ELISA to determine to which domain 791T/36 bound. The antigen was captured with either one of the anti-CD55 Mabs or 791T/36 and then detected with 791T/36. Thus, recognition of the antigen by the same antibody as the capture antibody would indicate that the

30   antibody is able to bind to two sites on the purified 791Tgp72. Conversely, absence of binding would indicate that the antibody has only one binding site on the antigen. In this way, antibodies can be mapped to the

different domains of CD55. Plates were coated with anti-CD55 antibodies 220 (SUSHI domain 1), 110 (SUSHI domain 2), BRIC 216 (SUSHI domain 3) and left at 4°C overnight. The plates were washed three times with PBS-Tween and

5   blocked with BSA (1%) for 1 hr at room temperature. The plates were washed three times, then purified 791Tgp72 antigen (25ng) was added. After 1 hr at room temperature, the plates were washed three times and biotinylated mAb 791T/36 (500ng per well) was added.

10   Following incubation at room temperature for 1 hr, and washing three times, streptavidin-HRPO diluted 1:1000 was added for a further 1 hr. After a further six washes, the plates were developed and read at 405 nm.

       Figure 7 shows that the 791Tgp72 antigen captured by

15   monoclonal antibodies which bound to SUSHI domains 1 and 3 could be detected by 791T/36 biotin. Interestingly, capture of 791Tgp72 by mAb 110, which was raised against SUSHI domain 2, or 791T/36 could not be detected by 791T/36 biotin, suggesting that 791T/36 must bind near

20   SUSHI domain 2.

       The anti-CD55 antibodies were tested in a competition assay for their ability to inhibit the binding of 791T/36 to 791T cells. The inhibition of 791T/36 binding would indicate that the competing

25   antibody bound to a similar or shared antigenic site on the 791Tgp72 molecule.

       791T cells (2 x 10^5) were mixed with different amounts of cold anti-CD55 monoclonal antibodies at 37°C for 30 minutes prior to adding mAb 791T/36 FITC (0.1 μg).

30   After 1 hr at 37°C, the cells were washed two times with RPMI 1640 medium, fixed and measured by flow cytofluorometry.

       Table 4 shows that in the competition binding assay

36

of anti-CD55 with 791T/36 FITC, only cold mAb 791T/36
inhibited the binding of labelled 791T/36. These results
suggest that although 791T/36 binds at or near SUSHI
domain 2, it binds at a distinct site to monoclonal
antibody 110.

Example 10

CHO Transfections with CD55/CD46 Chimeric Proteins

In order to ascertain the domain to which 791T/36
binds, a number of chimeric constructs were made
comprising CD46, a membrane bound complement control
protein with similar structure to CD55, i.e. contains
four SUSHI domains but those domains are distinct to
those of CD55. The constructs were produced by Dr Dale
Cristiansen (Austen Research Centre, Victoria,
Australia).   The constructs tested were:

(1)   CD46 (CD55 3); CD46 with SUSHI domain 3 substituted
      with that of CD55.

(2)   CD46 (CD55 4); CD46 with SUSHI domain 4 susbtituted
      with that of CD55.

(3)   CD46 (CD55 3/4); CD46 with SUSHI domains 3/4
      substituted with those of CD55.

(4)   CD46 (CD55 1/2); CD46 with SUSHI domains 1/2
      substituted with those of CD55.

Only CHO cells transfected with constructs
containing CD55 SUSHI2 showed significant binding to
791T/36 monoclonal antibody (Table 1).

Example 11

Anti-Idiotypic Antibodies

A human (105AD7) and a mouse (730) anti-idiotypic
antibodies which bind at the antigen combining site of
791T/36 have been produced. A competition assay was used

37

to assess if these anti-idiotypic antibodies could also
bind to the other anti-CD55 antibodies. 791T cells
$(2x10^5)$ were mixed with anti-CD55 (0.1 μg) and varying
amounts of 105AD7 or 730 at 37°C for 1hr. The cells were
5   washed two times with RPMI 1640 medium prior to the
addition of rabbit anti-mouse FITC (1:100) for a further
1hr. The cells were washed two times with RPMI 1640
medium, fixed and measured by flow cytofluorometry.

The results in tables 5 and 6 indicate the binding
10   of 791T/36 to 791T cells decreased when increasing
concentration of mAb 105AD7 or 730 were added. In
contrast, no loss in binding of the other anti-CD55 was
seen in the presence of either anti-idiotype. These
results add support to the conclusion that 791T/36 is a
15   unique anti-CD55 monoclonal antibody.

The anti-idiotypic antibodies both stimulate humoral
and cellular responses against cells which express
791Tgp72 antigen suggesting that they can mimic the
antigen. Comparison of the amino acid sequences of both
20   anti-idiotypes with CD55 show areas of homology with both
CDRH3 regions of the antibodies and distinct regions of
SUSHI domain 2.
For 105AD7:
CDR L1 - homology 7/9 amino acids with SUSHI1 83-93.
25   CDH H3 - homology 5/7 amino acids with SUSHI2 151-158.
For 730:
CDR L1 - homology 6/9 amino acids with SUSHI1 83-93.
CDH H3 - homology 5/7 amino acids with SUSHI2 121-128.

30

Example 12
Western Blotting
To confirm that 791T/36 could immunoprecipitate CD55

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from normal cells, erythrocytes (2×10^9) were washed two times with PBS and solubilized by NP-40 (1%, 10 ml). After centrifugation at 3000 rpm for 10 minutes, the supernatant was removed to a clean tube for

5 centrifugation ($100,000g \times 30$ min). 10 μl of erythrocyte supernatant (equal to 2×10^6 of erythrocytes) and purified 791Tgp72 antigen (200 ng) was loaded onto SDS-PAGE at non-reducing condition as described previously. Proteins were transferred to nitrocellulose membrane and blocked

10 with PBS containing BSA (1%) for 1 hr at room temperature (RT). After two times wash with PBS-Tween (0.1%), primary antibody was added for 1 hr at RT. The blots were washed two times and rabbit anti-mouse conjugate diluted 1:1000 was added. Following 1 hr incubation and

15 extensive washing, the blots were developed by ECL system.

Detecting the 791Tgp72 antigen from erythrocytes and 791T cells by Western blotting indicated some differences. Only one band at 72 kDa was found on

20 erythrocytes whereas two bands of 72 and 66 kDa exist on 791T cells (Figure 8).

Treatment of the 72 kDa and 66 kDa bands with neuraminidase (which removes sialic acid residues from glycoproteins) yielded in each case a band of 55 kDa,

25 suggesting that the 72 kDa and 66 kDa proteins are glycosylation variants of each other.

Example 13

Clone and DNA Sequence

30 To confirm the identity of 791Tgp72, the gene encoding this protein was cloned and sequenced. Total cellular RNA was isolated by the guanidine isothiocyanate method from 791T cells (4×10^7) grown in monolayers.

-39-

First-strand cDNA synthesis was carried out using Ready-To-Go First-Strand Kit (Pharmacia Biotech, UK). Primers based on the N-terminal protein sequence obtained from the 72 and 66 KDa bands were generated;

5   Pep 5':   GACTGTGGCCTTCCCCCAG

C-CD55-5':   AAAATGACCGTCGCGCGGCCG

C-CD55-3':   CTAAGTCAGCAAGCCCATGGT

B-CD55-5':   GAATACTGCAGATGACCGTCGCGCGGCCG

B-CD55-3':   CCTACGAATTCTAAGTCAGCAAGCCCATGG

10   FL-CD55-3': ATGTGATTCCAGGACTGCC

FL-CD55-5': TGGGCGTAGCTGCGACTCG

These primers were designed for the following:

       C-CD55: Cloning and expression in eukaryotic cells

15   from the recognised start codon to the stop codon of native CD55.

       B-CD55: Cloning and expression into a bacterial expression vector in order to generate protein for purification. The sequences include the addition of a 5'

20   EcoRI site and a 3' PstI site.

       FL-CD55: were designed for cloning of the recognised coding region of CD55 and 200 bp of the 3' untranslated region. This should allow the cloning of potential splice variants that occur in the 3' end of the antigen.

25   791Tgp72 PCRs were set up with first strand cDNA , the primers used were mixes of the primer sets outlined above. The samples were placed in a thermal cycler, the following profile was used (hot start at 94°C for 2 minutes; denaturation at 94°C for 30 seconds, 55°C for 45

30   seconds, 72°C for 90 seconds, repeat for a total 30 cycles). PCR products were cloned into modified pBluescript SK-vector. Positive clones were checked by PCR using vector specific primers and the positive DNA

40

plasmids sequenced on an ABI automated sequencer.

The initial cloning experiments resulted in products generated from PEP5' and either CCD553', BCD553' or FLCD553'. The results of this sequencing revealed there

5   to be no difference in sequence between the cloned products and the full reported sequences of CD55 (figure 9). The translated amino acid sequence of CD55 is set out in figure 10.

Recently full length versions of the PCR generated

10   CD55 products have been cloned using FLCD555' and either FLCD553', CCD553' or BCD553'.

Discussion

Interest in the use of 791Tgp72 as a target for

15   immunotherapy arose initially by the demonstration that this antigen was expressed by the majority of osteosarcomas, colorectal, gastric and ovarian tumours. The tumour specificity of 791Tgp72 was also emphasised by extensive clinical imaging studies with radiolabelled

20   anti-791Tgp72 monoclonal antibody 791T/36, in the detection of primary and metastatic colorectal cancer, osteosarcoma, breast and ovarian cancer. The results shown herein relate to the first isolation of 791Tgp72 and the use of this antigen or a related family member

25   CD55 as a cancer vaccine.

From the prior art, CD55 is a very surprising target for T-cell immunity as it is expressed on essentially all haematopoietic cells and on endothelial and epithelial tissues, including the vascular endothelium,

30   gastrointestinal tract, genitourinary tract, central nervous system, and extracellular matrix. 791T/36 binds weakly to erythrocytes and it may be that this has been advantageous in the clinical imaging studies. 791T/36

antibody may have bound weakly to erythrocytes which upon
passage through the tumour have allowed transfer of the
antibody to 791Tgp72 to which it binds with higher
affinity. CD55 was initially purified based on its
ability to accelerate the decay of the classical pathway
C3 convertase, C4b2a. It carries out the same function
with respect to the alternative pathway C3 convertase,
C3bBb, but does not have any cofactor activity for the
factor I-mediated proteolytic degradation of C3b or C4b.
So CD55 protects the cell from complement-mediated lysis
at the C3 convertase step.

Normal human tissues express membrane-associated
complement inhibitory proteins that protect these tissues
from damage by autologous complement. To determine
whether neoplasms also express these proteins prior
investigators have examined the distribution of CD55
(DAF), CD59 (protectin) and CD46 (membrane cofactor
protein) in frozen samples of human breast, colon,
kidney, and lung carcinomas and in adjacent non-
neoplastic tissues. Difference between normal tissues
and the corresponding neoplasms were observed, with loss
or gain of expression of one or more inhibitors. Some
tumours expressed only one inhibitor whether others
expressed different combinations of two or three
inhibitors. Colon carcinomas, by contrast, expressed all
inhibitors. The results demonstrate that most
carcinomas, with the exception of small cell carcinomas
of the lung, do express one or more complement inhibitors
at a level likely to inhibit complement-mediated cellular
damage. Other tumour tissues, such as ovarian and
gastrointestinal tumour cells, were also checked. The
surface expression level of CD55 varied, and correlation
with the vulnerability of the cells to C-mediated lysis.

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Thus, the expression of C regulators on malignant cells
may constitute a tumour escape mechanism, and is a
critical parameter to be examined when mAb therapy is
being considered. Furthermore, expression of CD55 on
target cells makes them resistant to lysis by natural
killer cells. Many tumours escape T-cell recognition by
loss of MHC molecules, however this makes them
susceptible to NK killing. Over-expression of CD55 which
inhibits NK lysis is therefore an obvious advantage.

The extensive expression of CD55 on normal cells,
its role in protecting cells from complement and NK lysis
makes a very unlikely target for T-cell immunotherapy.
However, clinical trials with 105AD7 which mimics an
epitope on CD55 are showing that it can stimulate
excellent T-cell responses. 791Tgp72 does however show
some differences from CD55. Two bands are precipitated
from tumour cells whereas only one band is seen in
erythrocytes. Although the anti-CD55 antibodies can
precipitate the 791Tgp72 from tumour cells the yield is
much lower than is observed with 791T/36. This is
reflected in the cell binding assays where 791T/36 shows
strongest binding to tumour cells whereas the anti-CD55
monoclonal antibody BRIC 216 binds better to
erythrocytes. Different forms of CD55 have been isolated
from tissue such as erythrocytes, urine and tears
(Nakano et al, 1991; Sugita et al, 1988; Seya et al,
1995). CD55-A (63kDa) and CD55-B (55kDa) from
erythrocytes do not appear to have a GPI anchor. CD55-U2
(60-80kDa) in urine is thought to be inactive. The
existence of a human splice variant of CD55 has been
suggested but the putative protein has never been
isolated. Furthermore, new functions other than
complement decay have been suggested. Activated T-cells

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which have been crosslinked with anti-CD55 monoclonal
antibodies can induce T-cell proliferation and signal
transduction. It is unclear if this is related to the
recent observation that CD55 is the ligand for the CD97
receptor expressed on activated T-cells.

Whether there are different roles for CD55 or
different forms of CD55/791Tgp72 in tumour cells or
whether there is differential it remains an interesting
prospect to use a molecule which tumours over-express to
protect themselves from immune attack as a cancer
vaccine. The dichotomy being that if the cell fails to
express the molecule it is susceptible to complement
mediated and NK lysis and if it does express the antigen
it will be killed by CD55 specific T-cells.

44

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Table 1.

	Fluorescence of Mabs on chimeric transfectants		
Constructs	791T/36	1H4	E4.3
wt DAF	105	164	16
DAF 3	4	94	79
DAF 4	3	-	70
DAF 3/4	5	-	70
DAF 1/2	108	-	3

(a) 1H4 Mab binds to sushi domain 3 of DAF
(b) E4.3 Mab binds to sushi domain 1 of CD 46

Chimeric constructs were transfected into CHO cells and assayed for their binding of 791T/36, 1H4 and E4.3.

Table 2.

Indirect Immunofluorescence Assay Of Various Antibodies On DAF Positive And Negative Cells

Mean Linear Fluorescence			
Antibody	Erythrocytes	791T	HUVEC
110	52.71	1186.96	109.33
216	94.28	1776.41	172.52
791T/36	60.07	2373.89	146.96
Rabbit anti-mouse FITC	26.68	27.88	55.55
708	24.11	36.42	47.38

Monoclonal antibodies to DAF (110, 216) and 791Tgp72 (791T36) were used to label a range of cells for 1 hour at 4°C. Cells were then incubated with FITC-labelled rabbit anti mouse antibody and read by FACS. Analysis was also carried out using Mab 708 as a negative control and with Rabbit anti-mouse FITC alone.

Table 3.

Binding Of 791T/36, Control IgG2b (708) And Anti-DAF Antibodies To 791T Cells Following Treatment With PI-PLC At 1u/ml For 1 Hour.

Antibodies	PI-PLC (-)	PI-PLC(+)	% Inhibition
110	963.40	86.34	91
216	1101.51	208.83	82
791T/36	1991.35	178.6	91
708	29.79	25.1	14

Mean linear fluorescence readings of anti DAF (110, 216) Mabs and anti 791T/gp72 (791T/36) antibodies to phospholipase treated or untreated 791T cells. 708 Mab was used as a control antibody.

Table 4.

Competitive Binding Of 791T/36 FITC (0.1ug) To 791T Cells By Anti DAF Antibodies,
Unlabelled 791T/36 Or An Irrelevant IgG2b Mab Which Does Not Bind To 791T Cells

Cold Mab (ug)	708	220	110	216	791T/36	% Inhibition with 791T/36
0	453.82	450.96	459.65	502.11	473.21	0
0.1	426.73	505.04	488.02	456.44	335.41	30
0.5	451.60	499.91	493.05	496.46	162.38	66
1.0	454.20	455.08	497.82	509.80	103.47	79

FITC labelled 791T/36 antibody was incubated with various concentrations of the above unlabelled
antibodies. Only unlabelled 791T/36 was able to inhibit binding of the FITC labelled 791T/36. Mean
linear fluorescence readings are given.

Table 5.

Competition Assay Of 105AD7 With Various Anti-DAF Antibodies On 791T Cell

105AD7 (ug)	708	110	216	791T/36
0	15.03	489.87	803.19	912.46
0.05	14.84	528.65	701.71	1089.17
0.1	17.24	388.11	783.55	912.35
0.5	14.72	533.11	607.61	726.25
1.0	16.14	512.53	626.31	570.13
5.0	20.85	370.53	562.24	53.07

Anti-DAF antibodies (110, 216) and anti 791T/gp72 (791T/36) were added to 791T cells in the presence of increasing concentrations of 105AD7 anti-idiotypic antibody, which specifically recognises the binding site of 791T/36. Cells were incubated for 1 hour at 37°C then for a further hour in the presence of FITC-labelled Rabbit anti-mouse. Cells were analysed by FACS. 708 Mab was used as a negative control. The results indicate that only 791T/36 was inhibited by 105AD7. Mean linear fluorescence readings are given.

Table 6.

Competition Assay Of 730 With Various Anti-DAF Antibodies On 791T Cell

730 (ug)	708	110	216	791T/36
0	19.97	607	1356	1382
0.1	17.63	642	1185	1352
0.5	19.02	632	1158	983
1.0	19.31	620	1229	597
2.5	23.10	739	1212	89
5.0	32.54	640	1179	70

Anti-DAF antibodies (110, 216) and anti 791T/gp72 (791T/36) were added to 791T cells in the presence of increasing concentrations of Mab 730 anti-idiotypic antibody, which specifically recognises the binding site of 791T/36. Cells were incubated for 1 hour at 37°C then for a further hour in the presence of FITC-labelled Rabbit anti-mouse. Cells were analysed by FACS. 708 Mab was used as a negative control. The results indicate that only 791T/36 was inhibited by 730. Mean linear fluorescence readings are given.

CLAIMS:

1.   A cancer vaccine comprising a polypeptide of the
CD55 family, or a fragment or derivative of a polypeptide
of the CD55 family, wherein the vaccine is capable of
inducing an immune response in a patient.

2.   A cancer vaccine according to claim 1 wherein the
polypeptide of the CD55 family is, or the fragment or
derivative is of, 791Tgp72 antigen.

3.   A cancer vaccine according to claim 1 or claim 2
comprising full length 791Tgp72 antigen or CD55
polypeptide.

4.   A cancer vaccine according to any preceding claim
wherein the antigen, polypeptide, fragment or derivative
has part or all of the amino acid sequence of Fig. 10.

5.   A cancer vaccine according to any preceding claim
wherein the antigen, polypeptide, fragment or derivative
includes part or all of the amino acid sequence
consisting of amino acids 97-159 of Fig. 10.

6.   A cancer vaccine according to claim 5 wherein the
antigen, polypeptide, fragment or derivative includes a
sequence having at least five amino acids identical with
corresponding amino acids of a contiguous stretch of
seven amino acids contained within amino acids 121-128 or
151-158 of Fig. 10.

7.   A cancer vaccine according to any preceding claim
wherein the antigen, polypeptide, fragment or derivative

includes a sequence having at least six amino acids identical with corresponding amino acids of a contiguous stretch of nine amino acids contained within amino acids 83-93 of Fig. 10.

5

8.   A cancer vaccine according to any preceding claim comprising a fragment of at least five contiguous amino acids from a polypeptide of the CD55 family.

10   9.   A cancer vaccine according to claim 8 comprising a fragment of at least five contiguous amino acids from the amino acid sequence shown in Fig. 10.

10.   A cancer vaccine according to claim 8 or claim 9

15   wherein the fragment is of at least seven contiguous amino acids.

11.   A cancer vaccine according to claim 10 wherein the fragment is of at least nine contiguous amino acids.

20

12.   A cancer vaccine according to claim 11 wherein the fragment is of at least 13 contiguous amino acids.

13.   A cancer vaccine comprising a nucleic acid molecule

25   which encodes an antigen, polypeptide, fragment or derivative as specified in any preceding claim, wherein the vaccine is capable of inducing an immune response in a patient.

30   14.   A cancer vaccine according to claim 13 having part or all of a nucleic acid sequence as shown in Fig. 10 or Fig. 11.

15. A cancer vaccine according to any preceding claim wherein the immune response is one or more of a T-helper cell response, a cytotoxic T-cell response and a NK cell response.

16. A cancer vaccine according to any preceding claim which is capable of inducing an immune response against CD55 or 791gp72 as expressed by cancer cells.

17. A cancer vaccine according to claim 16 wherein the immune response has greater affinity for cancer cells than for non-cancerous cells.

18. The use of an antigen, polypeptide, fragment, derivative or a nucleic acid molecule as specified in any preceding claim in the preparation of a medicament for the treatment of cancer.

19. A method of treating a patient having cancer, the method comprising administering to the patient a therapeutically effective amount of a cancer vaccine as defined in any one of claims 1 to 17.

20. Isolated and purified 791Tgp72 antigen.

21. Isolated and purified 791Tgp72 antigen according to claim 20 having the amino acid sequence shown in Fig. 10 and a molecular weight of approximately 66 kD.

22. Isolated and purified 791Tgp72 antigen according to claim 20 or claim 21 wherein the specificity of antibody binding to the said antigen, relative to antibody binding to the CD55 antigen as expressed on human red blood cells

55

and/or HUVEC cells, is greater for 791T/36 than for anti-CD55 antibody BRIC 216.

23.   Isolated and purified 791Tgp72 antigen according to any one of claims 20 to 22, as obtainable by:
       (a)   solubilising 791T cells in lysis buffer including 1% octyl-B-glucoside, pH 8.5 for 1 hour at 4°C;
       (b)   centrifuging the lysate at 13000 rpm x 10 min following 100,000 g x 30 min;
       (c)   adding the cleared lysate to Protein A sepharose coupled to 791T/36 affinity column;
       (d)   cycling the supernatant over the column at 0.3-0.4 ml/min;
       (e)   washing the column with 20ml 20 mM Tris-HCl pH 8.0 containing 0.3 M NaCl and 0.1% NP-40; and,
       (f)   eluting 791Tgp72 from the column in 5 column volumes of diethylamine pH 11.5 containing 0.5% NP-40 and neutralising the eluate with 1M Tris.

24.   A pharmaceutical composition comprising 791Tgp72 according to any one of claims 20 to 23 in combination with a pharmaceutically acceptable carrier.

25.   791Tgp72 according to any one of claims 20 to 23 for use in a method of medical treatment.

26.   A method for isolating 791Tgp72 antigen from cells expressing 791Tgp72, the method including the steps of:
       solubilising the cells with lysis buffer including octyl-glucoside; and
       treating the lysate using ultracentrifugation.

72K
66K

Lane	1	2	3	4	5	6	7	8
Biotinylation	+	+	+	+	+	+	+	+
791T/36	+	-	+	-	+	-	+	-
1143/B7	-	+	-	+	-	+	-	+
DTSSP	-	-	+	+	-	-	+	+
Solublisation	x	x	x	x	o	o	o	o

1 2 3 4 5 6 7 8
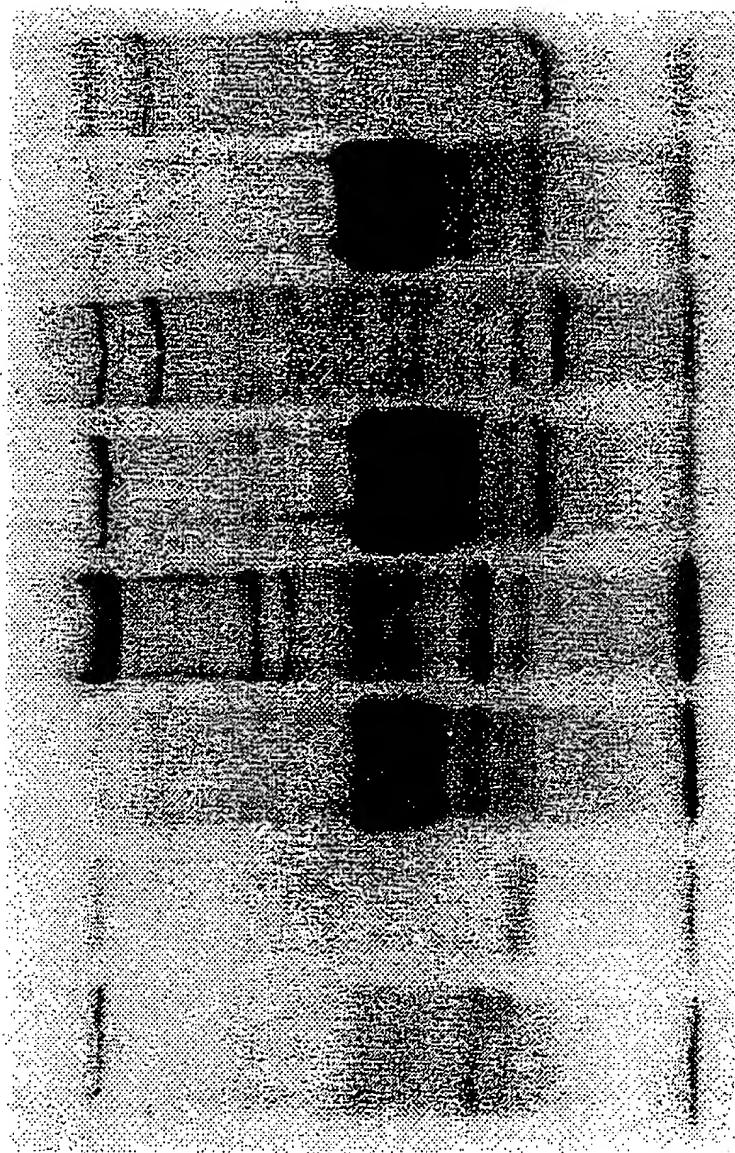
Fig. 1

Detergent

Glucoside	+	-	-	-	-	-
NP-40	-	+	-	-	-	-
TX-100	-	-	+	+	+	+

Centrifugation

13000 rpm	+	+	+	+	+	+
100, 000 g	+	+	+	-	+	+

blue-2   -   -   -   -   +   +

97.4 K

66 K

45 K

Fig. 2

Fig. 3

4/23
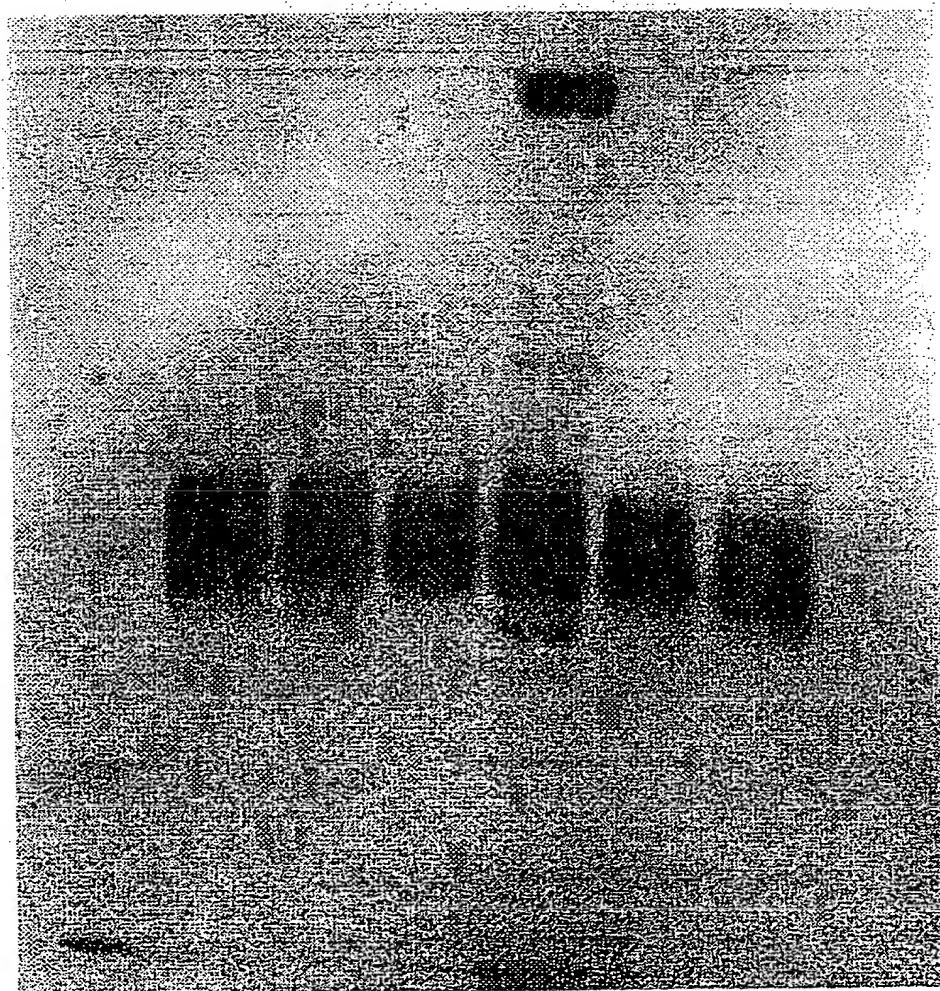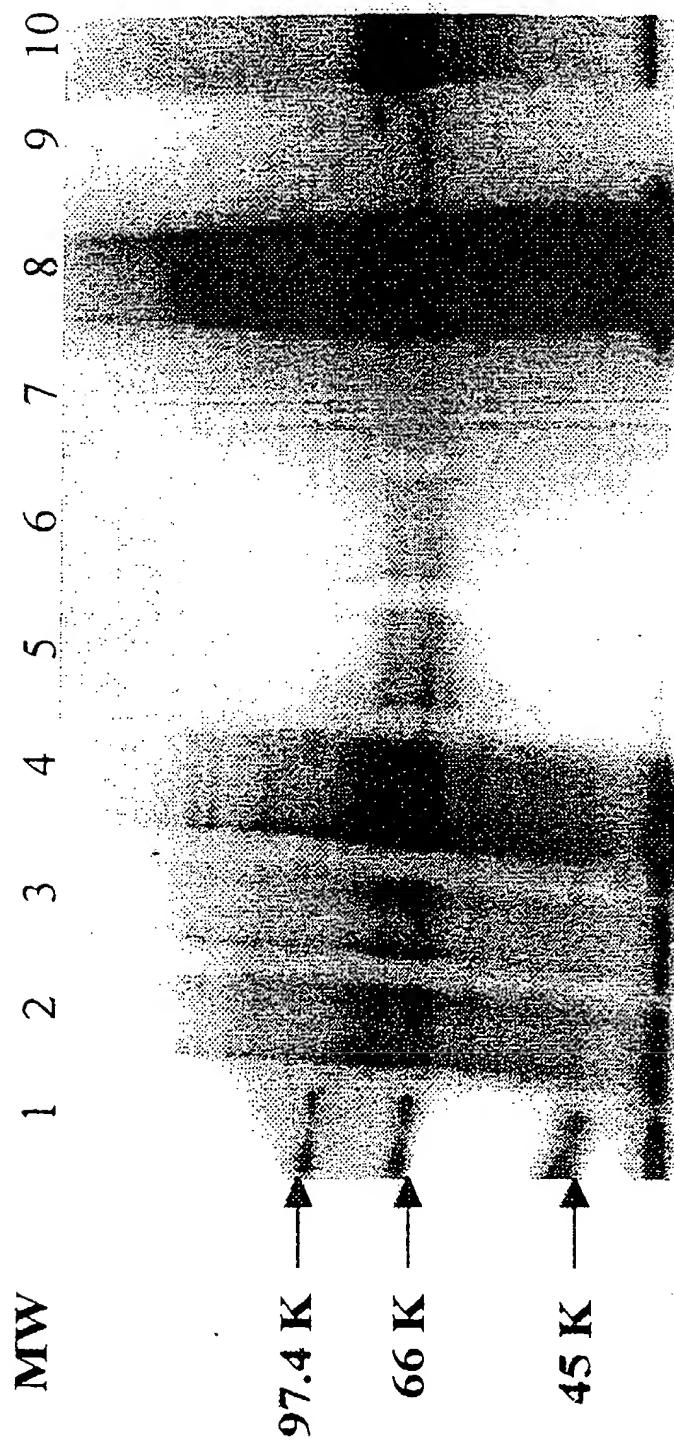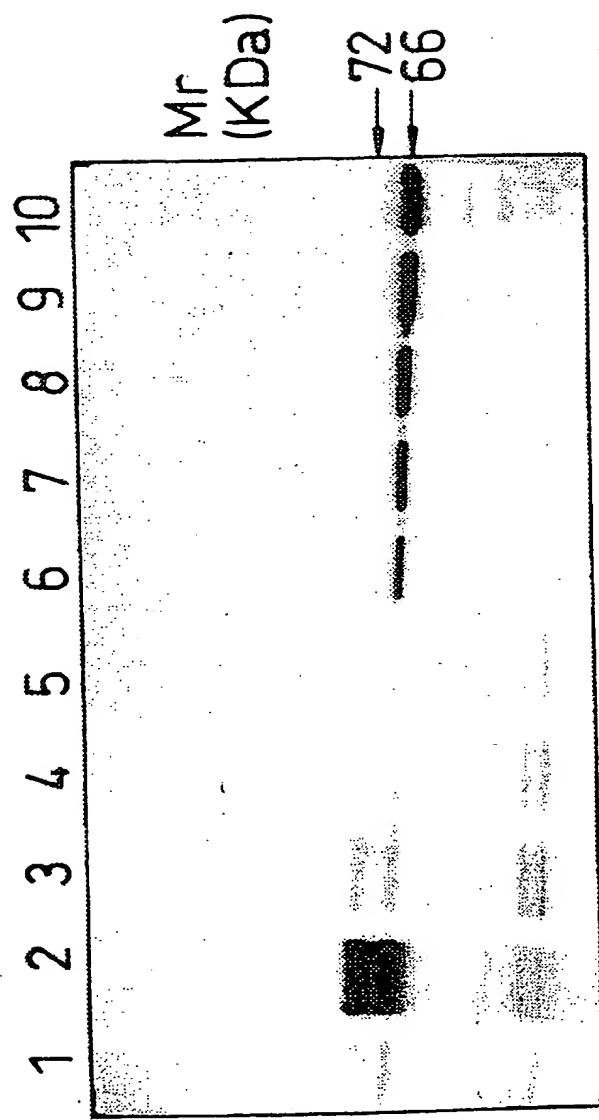
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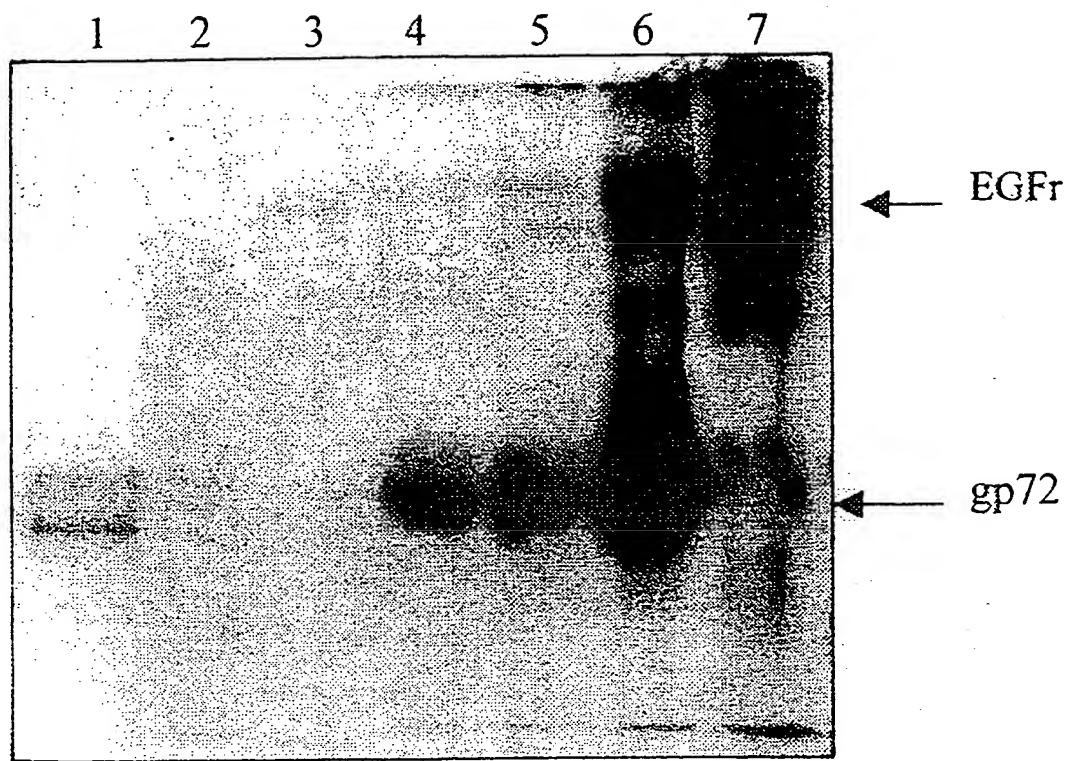
Fig. 5

6/23
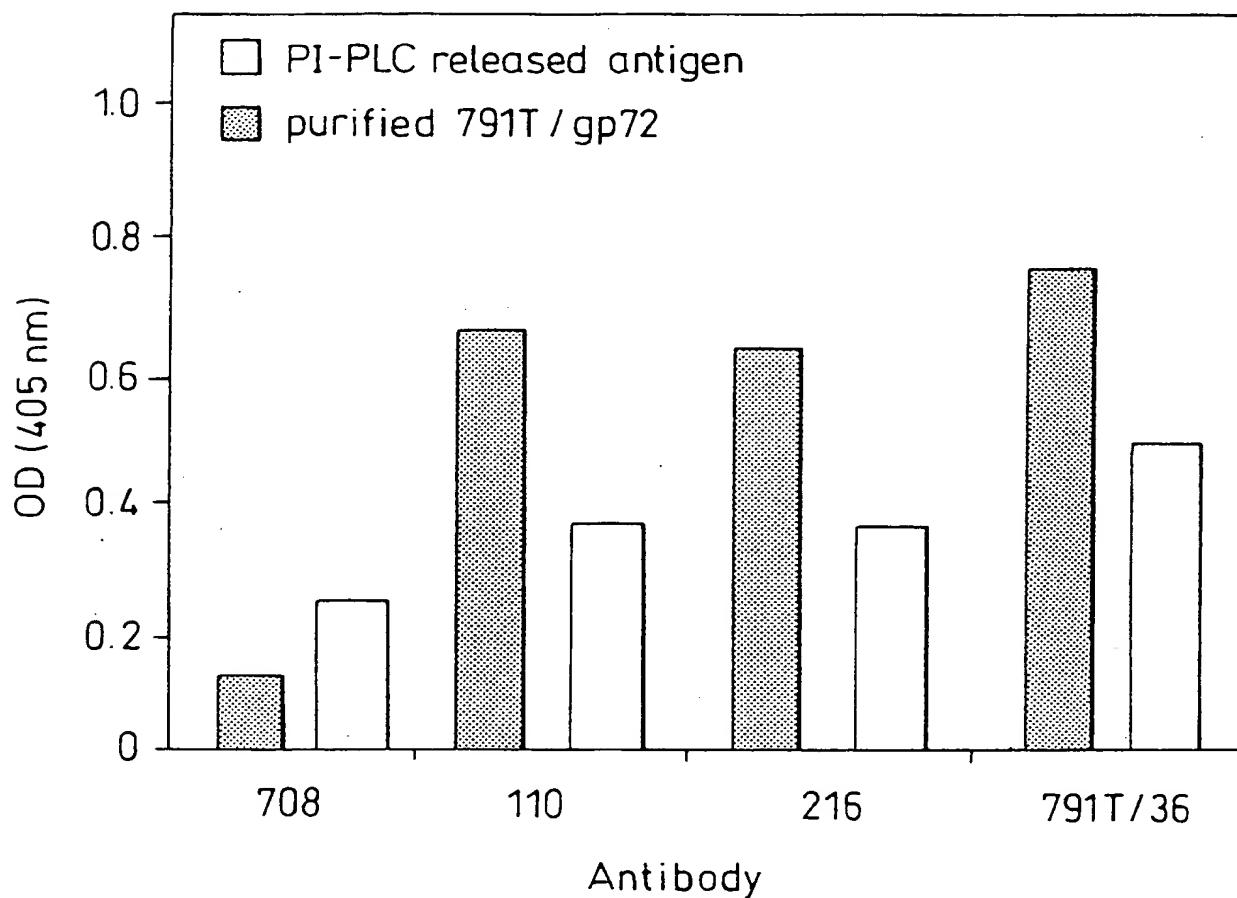
Fig. 6

7/23
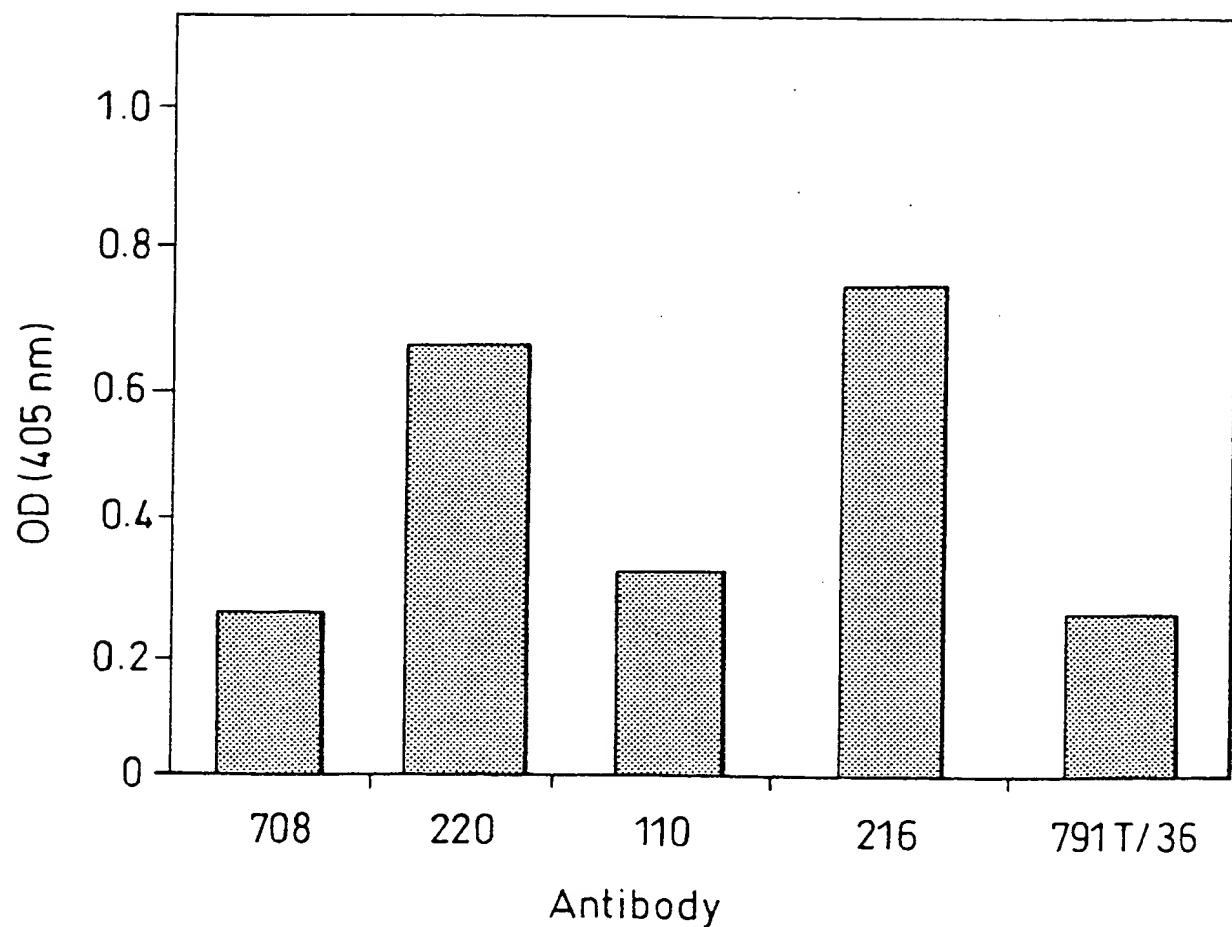
Fig. 7

8/23

Erythrocytes   791T cells

216   791T/36   708   791T/36   216   110   Antibodies

72 K
66 K

Fig. 8

```
                    10        20        30        40        50
                    |         |         |         |         |
5/P5            ------------------------------------------------
C-DAF.seq       CCGCTGGGGCGTAGCTGCGACTCGGGCGGAGTCCCGGCGGCCGTCCTTGTTCTAACCCG
RC of B/C  DAF  ------------------------------------------------

                    60        70        80        90       100       110
                    |         |         |         |         |         |
5/P5            ----------------------------------------------------------
C-DAF.seq       GCGGCGCCATGACCGTCGCGGCCGTGCCCGCGGCCGTGCCCCTCCTCGCCCTCCTCGGGGAG
RC of B/C  DAF  ----------------------------------------------------------

                   120       130       140       150       160       170
                    |         |         |         |         |         |
5/P5            ----------------------------------------------------------
C-DAF.seq       CTGCCCCGGCTGCTGCTGCTGGTGGTGTTGTGCCTGCCCGCCGTGTGGGGGTGACTGTG
RC of B/C  DAF  ----------------------------------------------------------
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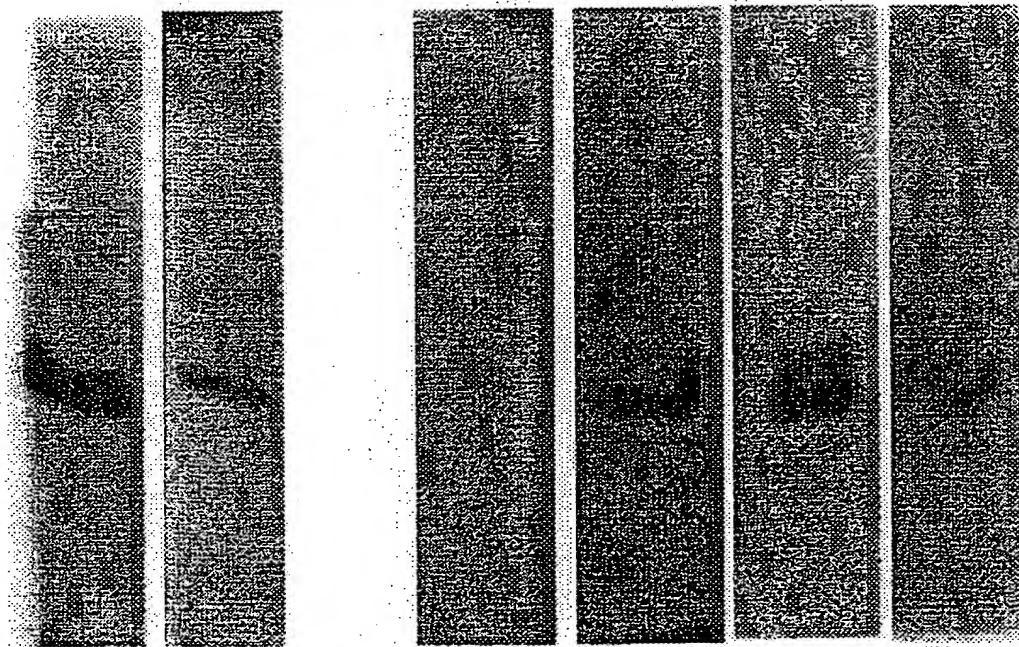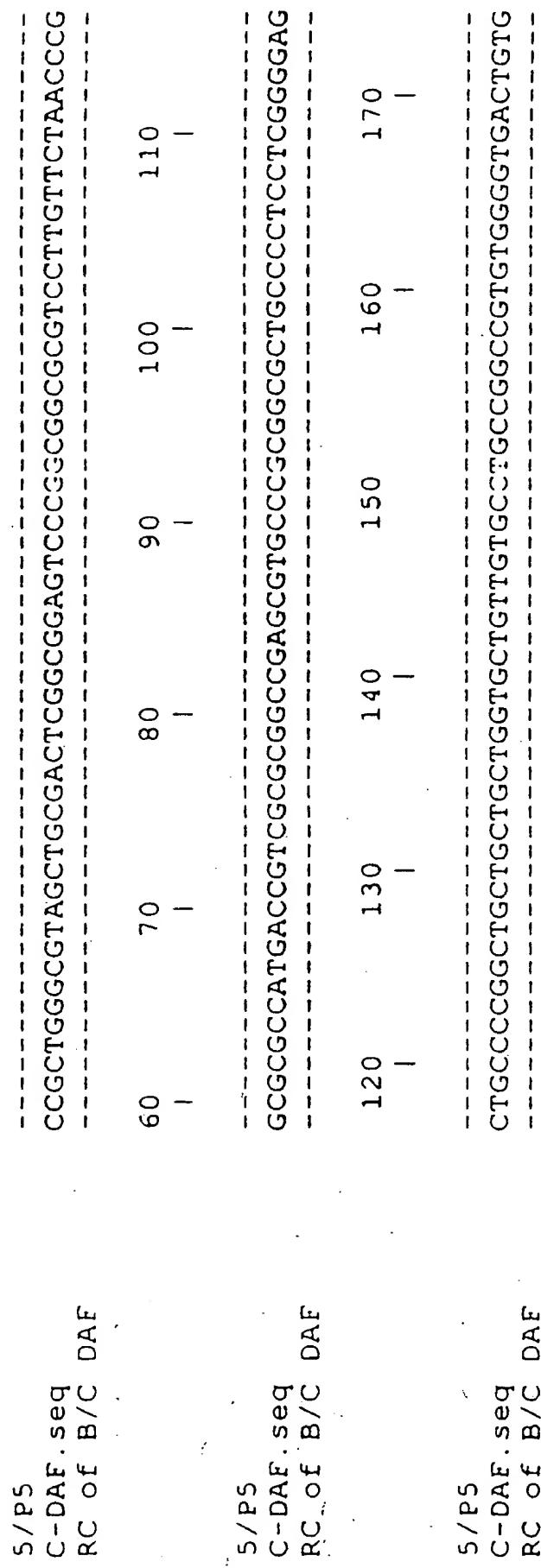
Fig. 9 (part 1 of 11)

```
        180    190    200    210    220    230
5/P5        ----------------------------------------------GTACAAGTTTCC
C-DAF.seq   GCCTTCCCCCAGATGTACCTAATGCCCAGCCAGCTTTGGAAGGCCGTACAAGTTTCC
RC of B/C DAF -------------------------------------------------------

        240    250    260    270    280    290
5/P5        CGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCCTGGCGAG
C-DAF.seq   CGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCCTGGCGAG
RC of B/C DAF -------------------------------------------------------

        300    310    320    330    340
5/P5        AAGGACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCA
C-DAF.seq   AAGGACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCA
RC of B/C DAF -------------------------------------------------------
```

Fig. 9 (part 2 of 11)

```
          350        360        370        380        390        400
           |          |          |          |          |          |
5/P5        ATCGTAGCTGCGAGGTGCCAACAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATAT
C-DAF.seq   ATCGTAGCTGCGAGGTGCCAACAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATAT
RC of B/C DAF ------------------------------------------------------------

          410        420        430        440        450        460
           |          |          |          |          |          |
5/P5        CACTCAGAATTATTTTCCAGTCGGTCGGTACTGTTGTGGAATATGAGTGCCGTCCAGGTTAC
C-DAF.seq   CACTCAGAATTATTTTCCAGTCGGTCGGTACTGTTGTGGAATATGAGTGCCGTCCAGGTTAC
RC of B/C DAF ------------------------------------------------------------

          470        480        490        500        510        520
           |          |          |          |          |          |
5/P5        AGAAGAGAACCTTCTCTATCACCAAAACTAACTTGCCTTCAGAATTTAAAATGGTCCA
C-DAF.seq   AGAAGAGAACCTTCTCTATCACCAAAACTAACTTGCCTTCAGAATTTAAAATGGTCCA
RC of B/C DAF ------------------------------------------------------------

          530        540        550        560        570        580
           |          |          |          |          |          |
5/P5        CAGCAGTCGAATTTTGTAAAAAGAAATCATGCCCTAATCCGGGAGAAATACGAAATGG
C-DAF.seq   CAGCAGTCGAATTTTGTAAAAAGAAATCATGCCCTAATCCGGGAGAAATACGAAATGG
RC of B/C DAF ------------------------------------------------------------
```

Fig. 9 (part 3 of 11)

```
              590       600       610       620       630
               |         |         |         |         |
5/P5           TCAGATTGATGTACCAGGTGGCATATTATTTGATGCAACCATCTCCTTCTCATGTAA
C-DAF.seq      TCAGATTGATGTACCAGGTGGCATATTATTTGGTGCAACCATCTCCTTCTCATGTAA
RC of B/C DAF  --------------------------TATTATTTGNTGCAACCATTCCTTTCATGTAA

              640       650       660       670       680       690
               |         |         |         |         |         |
5/P5           CACAGGGGTACAAATTATTGGCTCGACTTCTAGTTTTTGTCTTATTTCAGGCAGCTC
C-DAF.seq      CACAGGG-TACAAATTATTGGCTCGACTTCTAGTTTTTGTCTTATTTCAGGCAGCTC
RC of B/C DAF  CACANGG-TACAAATTATTGGCTCGACTTCTAGTTTTTGTCTTATTTCAGGCAGCTC

              700       710       720       730       740       750
               |         |         |         |         |         |
5/P5           TGTCCAGTGGAGTGACCCGTTGCCAGAATGCAGAGAAATTTA-TGTCCAGCACCACCA
C-DAF.seq      TGTCCAGTGGAGTGACCCGTTGCCAGAGTGCAGAGAAATTTATTGTCCAGCACCACCA
RC of B/C DAF  TGTCCAGTGGAGTGACCCGTTGCCAGAGTGCAGAGAAATTTATTGTCCAGCACCACCA
```

Fig. 9 (part 4 of 11)

```
          760       770       780       790       800       810
          |         |         |         |         |         |
5/P5        CAAATTGACA-TGGAATAATCCAGGGGAACGTTGACCATTATGGATATAAACG-----
C-DAF.seq   CAAATTGACAATGGAATAATTCAAGGGGAACGTGACCATTATGGATATAGACAGTCTG
RC of B/C DAF CAAATTGACAATGGAATAATTCAAGGGGAACGTGACCATTATGGATATAGACAGTCTG

          820       830       840       850       860       870
          |         |         |         |         |         |
5/P5        TAACGTATGCATGTAATAAAGGATTCACCATGATTGGAGAGCACTCTATTTATTGTAC
C-DAF.seq   TAACGTATGCATGTAATAAAGGATTCACCATGATTGGAGAGCACTCTATTTATTGTAC
RC of B/C DAF -------------------------------------------------------------

          880       890       900       910       920
          |         |         |         |         |
5/P5        TGTGAATAATGATGAAGGAGAGTGGCCCACCACCTGAATGCAGAGGAAAAATCT
C-DAF.seq   TGTGAATAATGATGAAGGAGAGTGGCCCACCACCTGAATGCAGAGGAAAAATCT
RC of B/C DAF -------------------------------------------------------------
```

Fig. 9 (part 5 of 11)

```
         930    940    950    960    970    980
          -      -      -      -      -      -
5/P5
C-DAF.seq      CTAACTTCCAAGGTCCCACCAACAGTTCAGAAACCTACCACCAGTAAATGTTCCAACTA
RC of B/C DAF  CTAACTTCCAAGGTCCCACCAACAGTTCAGAAACCTACCACCAGTAAATGTTCCAACTA
          990    1000   1010   1020   1030   1040
           -      -      -      -      -      -

5/P5
C-DAF.seq      CAGAAGTCTCCACCAACTTCTCAGAAAACCACCACCAAAACCACCACCAAATGCTCA
RC of B/C DAF  CAGAAGTCTCCACCAACTTCTCAGAAAACCACCACCAAAACCACCACCAAATGCTCA
          1050   1060   1070   1080   1090   1100
           -      -      -      -      -      -

5/P5
C-DAF.seq      AGCAACACGGAGTACACCTGTTCCAGGACAACCAAGCATTTCATGAAACACACCCCA
RC of B/C DAF  AGCAACACGGAGTACACCTGTTCCAGGACAACCAAGCATTTCATGAAACACACCCCA
          1110   1120   1130   1140   1150   1160
           -      -      -      -      -      -

5/P5
C-DAF.seq      AATAAAGGAAGTGGAACCACTTCAGGTACTACCGTCTTCTATCTGGGCACACGTGTT
RC of B/C DAF  AATAAAGGAAGTGGAACCACTTCAGGTACTACCGTCTTCTATCTGGGCACACGTGTT
```

Fig. 9 (part 6 of 11)

```
                1170      1180      1190      1200      1210
                 |         |         |         |         |
                -----|---------|---------|---------|---------|---------|---------
5/P5
C-DAF.seq       TCACGTTGACAGGTTGCTTGGGACGGCTAGTAACCATGGGCTTGCTGACTTAGCCAAA
RC of B/C  DAF  TCAC
                -----|---------|---------|---------|---------|---------|---------

                1220      1230      1240      1250      1260      1270
                 |         |         |         |         |         |
                -----|---------|---------|---------|---------|---------|---------
5/P5
C-DAF.seq       GAAGAGTTAAGAAGAAAATACACACAAGTATACAGACTGTTCCTAGTTTCTTAGACTT
RC of B/C  DAF
                -----|---------|---------|---------|---------|---------|---------

                1280      1290      1300      1310      1320      1330
                 |         |         |         |         |         |
                -----|---------|---------|---------|---------|---------|---------
5/P5
C-DAF.seq       ATCTGCATATTGGATAAAATAAATGCAATTGTGCTCTTCATTTAGGATGCTTTCATTG
RC of B/C  DAF
                -----|---------|---------|---------|---------|---------|---------
```

Fig. 9 (part 7 of 11)

```
                  1340      1350      1360      1370      1380      1390
                     |         |         |         |         |         |
5/P5
C-DAF.seq        TCTTTAAGATGTGTTAGGAATGTCAACAGAGCAAGGAGAAAAAGGCAGTCCTGGAAT
RC of B/C DAF

                  1400      1410      1420      1430      1440      1450
                     |         |         |         |         |         |
5/P5
C-DAF.seq        CACATTCTTAGCACACCTACACCTCTTGAAAATAGAACAACTTGCAGAATTGAGAGTG
RC of B/C DAF

                  1460      1470      1480      1490      1500
                     |         |         |         |         |
5/P5
C-DAF.seq        ATTCCTTTCCTAAAAGTGTAAGAAAGCATAGAGATTTGTTCGTATTTAGAATGGGATC
RC of B/C DAF
```

Fig. 9 (part 8 of 11)

```
        1510      1520      1530      1540      1550      1560
5/P5
C-DAF.seq   ACGAGGAAAAGAGAGGAAAGTGATTTTTTTCCACAAGATCTGTAATGTTATTTCCAC
RC of B/C DAF

        1570      1580      1590      1600      1610      1620
5/P5
C-DAF.seq   TTATAAAGGAAATAAAAAATGAAAACATTATTGGATATCAAAAGCAAATAAAAACC
RC of B/C DAF

        1630      1640      1650      1660      1670      1680
5/P5
C-DAF.seq   CAATTCAGTCTCTTCTAAGCAAAATTGCTAAAGAGAGATGAACCACATTATAAAGTAA
RC of B/C DAF

        1690      1700      1710      1720      1730      1740
5/P5
C-DAF.seq   TCTTTGGCTGTAAGGCATTTTCATCTTTCCTTCGGGTTGGCAAAATATTTAAAGGTA
RC of B/C DAF
```

Fig. 9 (part 9 of 11)

Fig. 9 (part 10 of 11)

5/P5
C-DAF.seq
RC of B/C DAF

1750   1760   1770   1780   1790
AAACATGCTGGTGAACCAGGGGTGTTGATGGTGATAAGGGAGGAATATAGAATGAAAG

1800   1810   1820   1830   1840   1350
ACTGAATCTTCCTTTGTTGCACAAATAGAGTTTGGAAAAAGCCTGTGAAAGGTGTCTT

1860   1870   1880   1890   1900   1910
CTTTGACTTAATGTCTTTAAAAGTATCCAGAGATACTACAATATTAACATAAGAAAAG

1920   1930   1940   1950   1960   1970
ATTATATATTATTTCTGAATCGAGATGTCCATAGTCAAATTTGTAAATCTTTATTCTTT

5/P5
C-DAF.seq
RC of B/C DAF

```
                                                                    1980      1990                          2000            2010            2020            2030
                                                                     I         I                             I               I               I               I
5/P5
C-DAF.seq                                                           TGTAATATATTTATTTATATTATTTATTTATGACAGTGAACATTCTGATTTTACACATGTAAAAC
RC of B/C DAF

                                                                        2040              2050               2060            2070            2080
                                                                         I                 I                   I               I               I
5/P5
C-DAF.seq                                                           AAGAAAAGTTGAAGAAGATATGTGAAGAAAAATGTATTTTCCTAAATAGAAATAAAT
RC of B/C DAF

                                                                    2090       2100
                                                                     I          I
5/P5                                                                        ----------T
C-DAF.seq                                                           GATCCCCATTTTTGGT
RC of B/C DAF
```

Fig. 9 (part 11 of 11)

```
1                                                          CC GCT GGG CGT AGC

15   TGC GAC TCG GCG GAG TCC CGG CGG CGC GTC CTT GTT CTA ACC CGG CGC GCC
     |<-- Leader sequence
     Met Thr Val Ala Arg Pro Ser Val Pro Ala Ala Leu Pro Leu Leu Gly Glu  17
66   ATG ACC GTC GCG CGG CCG AGC GTG CCC GCG GCG CTG CCC CTC CTC GGG GAG

     Leu Pro Arg Leu Leu Leu Leu Val Leu Leu Cys Leu Pro Ala Val Trp Gly  34
117  CTG CCC CGG CTG CTG CTG CTG GTG CTG TTG TGC CTG CCG GCC GTG TGG GGT
     <- sushi 1
     |<-- mature sequence (first 16 amino acids sequenced)
     Asp Cys Gly Leu Pro Pro Asp Val Pro Asn Ala Gln Pro Ala Leu Glu Gly  51
168  GAC TGT GGC CTT CCC CCA GAT GTA CCT AAT GCC CAG CCA GCT TTG GAA GGC

     Arg Thr Ser Phe Pro Glu Asp Thr Val Ile Thr Tyr Lys Cys Glu Glu Ser  68
219  CGT ACA AGT TTT CCC GAG GAT ACT GTA ATA ACG TAC AAA TGT GAA GAA AGC

     Phe Val Lys Ile Pro Gly Glu Lys Asp Ser Val Ile Cys Leu Lys Gly Ser  85
270  TTT GTG AAA ATT CCT GGC GAG AAG GAC TCA GTG ATC TGC CTT AAG GGC AGT
                                        sushi 1 ->|    |sushi 2
     Gln Trp Ser Asp Ile Glu Glu Phe Cys Asn Arg Ser Cys Glu Val Pro Thr 102
321  CAA TGG TCA GAT ATT GAA GAG TTC TGC AAT CGT AGC TGC GAG GTG CCA ACA

     Arg Leu Asn Ser Ala Ser Leu Lys Gln Pro Tyr Ile Thr Gln Asn Tyr Phe 119
372  AGG CTA AAT TCT GCA TCC CTC AAA CAG CCT TAT ATC ACT CAG AAT TAT TTT

     Pro Val Gly Thr Val Val Glu Tyr Glu Cys Arg Pro Gly Tyr Arg Arg Glu 136
423  CCA GTC GGT ACT GTT GTG GAA TAT GAG TGC CGT CCA GGT TAC AGA AGA GAA

     Pro Ser Leu Ser Pro Lys Leu Thr Cys Leu Gln Asn Leu Lys Trp Ser Thr 153
474  CCT TCT CTA TCA CCA AAA CTA ACT TGC CTT CAG AAT TTA AAA TGG TCC ACA
                     sushi 2->|          |<- sushi 3
     Ala Val Glu Phe Cys Lys Lys Lys Ser Cys Pro Asn Pro Gly Glu Ile Arg 170
525  GCA GTC GAA TTT TGT AAA AAG AAA TCA TGC CCT AAT CCG GGA GAA ATA CGA

     Asn Gly Gln Ile Asp Val Pro Gly Gly Ile Leu Phe Gly Ala Thr Ile Ser 187
576  AAT GGT CAG ATT GAT GTA CCA GGT GGC ATA TTA TTT GGT GCA ACC ATC TCC

     Phe Ser Cys Asn Thr Gly Tyr Lys Leu Phe Gly Ser Thr Ser Ser Phe Cys 204
627  TTC TCA TGT AAC ACA GGG TAC AAA TTA TTT GGC TCG ACT TCT AGT TTT TGT
                                                           sushi 3 ->|
     Leu Ile Ser Gly Ser Ser Val Gln Trp Ser Asp Pro Leu Pro Glu Cys Arg 221
678  CTT ATT TCA GGC AGC TCT GTC CAG TGG AGT GAC CCG TTG CCA GAG TGC AGA
              |<- sushi 4
     Glu Ile Tyr Cys Pro Ala Pro Pro Gln Ile Asp Asn Gly Ile Ile Gln Gly 238
729  GAA ATT TAT TGT CCA GCA CCA CCA CAA ATT GAC AAT GGA ATA ATT CAA GGG
```

Fig. 10 (part 1 of 2)

```
        Glu Arg Asp His Tyr Gly Tyr Arg Gln Ser Val Thr Tyr Ala Cys Asn Lys 255
    780 GAA CGT GAC CAT TAT GGA TAT AGA CAG TCT GTA ACG TAT GCA TGT AAT AAA

        Gly Phe Thr Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp 272
    831 GGA TTC ACC ATG ATT GGA GAG CAC TCT ATT TAT TGT ACT GTG AAT AAT GAT
                                                     sushi 4->|
        Glu Gly Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Lys Ser Leu Thr 289
    885 GAA GGA GAG TGG AGT GGC CCA CCA CCT GAA TGC AGA GGA AAA TCT CTA ACT

        Ser Lys Val Pro Pro Thr Val Gln Lys Pro Thr Thr Val Asn Val Pro Thr 306
    933 TCC AAG GTC CCA CCA ACA GTT CAG AAA CCT ACC ACA GTA AAT GTT CCA ACT

        Thr Glu Val Ser Pro Thr Ser Gln Lys Thr Thr Thr Lys Thr Thr Thr Pro 323
    984 ACA GAA GTC TCA CCA ACT TCT CAG AAA ACC ACC ACA AAA ACC ACC ACA CCA

        Asn Ala Gln Ala Thr Arg Ser Thr Pro Val Ser Arg Thr Thr Lys His Phe 340
   1035 AAT GCT CAA GCA ACA CGG AGT ACA CCT GTT TCC AGG ACA ACC AAG CAT TTT

        His Glu Thr Thr Pro Asn Lys Gly Ser Gly Thr Thr Ser Gly Thr Thr Arg 357
   1086 CAT GAA ACA ACC CCA AAT AAA GGA AGT GGA ACC ACT TCA GGT ACT ACC CGT

        Leu Leu Ser Gly His Thr Cys Phe Thr Leu Thr Gly Leu Leu Gly Thr Leu 374
   1137 CTT CTA TCT GGG CAC ACG TGT TTC ACG TTG ACA GGT TTG CTT GGG ACG CTA

        Val Thr Met Gly Leu Leu Thr Stop                                    381
   1188 GTA ACC ATG GGC TTG CTG ACT TAG CCAAAGAAGAGTTAAGAAG

   1231 AAAATACACACAAGTATACAGACTGTTCCTAGTTTCTTAGACTTATCTGCATATTGGATAAAATAAA
   1298 TGCAATTGTGCTCTTCATTTAGGATGCTTTCATTGTCTTTAAGATGTGTTAGGAATGTCAACAGAGC
   1365 AAGGAGAAAAAAGGCAGTCCTGGAATCACATTCTTAGCACACCTACACCTCTTGAAAATAGAACAAC
   1432 TTGCAGAATTGAGAGTGATTCCTTTCCTAAAAGTGTAAGAAAGCATAGAGATTTGTTCGTATTTAGA
   1499 ATGGGATCACGAGGAAAAGAGAAGGAAAGTGATTTTTTTCCACAAGATCTGTAATGTTATTTCCACT
   1566 TATAAAGGAAATAAAAAATGAAAAACATTATTTGGATATCAAAAGCAAATAAAAACCCAATTCAGTC
   1633 TCTTCTAAGCAAAATTGCTAAAGAGAGATGAACCACATTATAAAGTAATCTTTGGCTGTAAGGCATT
   1700 TTCATCTTTCCTTCGGGTTGGCAAAATATTTTAAAGGTAAAACATGCTGGTGAACCAGGGGTGTTGA
   1767 TGGTGATAAGGGAGGAATATAGAATGAAAGACTGAATCTTCCTTTGTTGCACAAATAGAGTTTGGAA
   1834 AAAGCCTGTGAAAGGTGTCTTCTTTGACTTAATGTCTTTAAAAGTATCCAGAGATACTACAATATTA
   1901 ACATAAGAAAAGATTATATATTATTTCTGAATCGAGATGTCCATAGTCAAATTTGTAAATCTTATTC
   1968 TTTTGTAATATTTATTTATATTTATTTATGACAGTGAACATTCTGATTTTACATGTAAAACAAGAAA
   2035 AGTTGAAGAAGATATGTGAAGAAAAATGTATTTTTCCTAAATAGAAATAAATGATCCCATTTTTTGG
   2102 T
```

Fig. 10 (part 2 of 2)

```
  1    TTTAAACGGGCCCTCTAGACTCGAGCGGCCGCTGCCCATCTTGTCGTCGTCGTCCTTGTAGTCG
 65    TGCATGTGGTGGTGGTGGTGGTGGTTAACCATGGTGGCGGGCCGCCACTGTGCTGGATATCTGCAGA
132    ATTCGATGGGCGTAGCTGCGACTCGGCGGAGTCCCGGCGGCGCGTCCTTGTTCTAACCCGGCGCGCC
```

```
       Met Thr Val Ala Arg Pro Ser Val Pro Ala Ala Leu Pro Leu Leu Gly Glu  17
199    ATG ACC GTC GCG CGG CCG AGC GTG CCC GCG GCG CTG CCC CTC CTC GGG GAG

       Leu Pro Arg Leu Leu Leu Leu Val Leu Leu Cys Leu Pro Ala Val Trp Gly  34
250    CTG CCC CGG CTG CTG CTG CTG GTG CTG TTG TGC CTG CCG GCC GTG TGG GGT

       Asp Cys Gly Leu Pro Pro Asp Val Pro Asn Ala Gln Pro Ala Leu Glu Gly  51
301    GAC TGT GGC CTT CCC CCA GAT GTA CCT AAT GCC CAG CCA GCT TTG GAA GGC

       Arg Thr Ser Phe Pro Glu Asp Thr Val Ile Thr Tyr Lys Cys Glu Glu Ser  68
352    CGT ACA AGT TTT CCC GAG GAT ACT GTA ATA ACG TAC AAA TGT GAA GAA AGC

       Phe Val Lys Ile Pro Gly Glu Lys Asp Ser Val Ile Cys Leu Lys Gly Ser  85
403    TTT GTG AAA ATT CCT GGC GAG AAG GAC TCA GTG ATC TGC CTT AAG GGC AGT

       Gln Trp Ser Asp Ile Glu Glu Phe Cys Asn Arg Ser Cys Glu Val Pro Thr 102
454    CAA TGG TCA GAT ATT GAA GAG TTC TGC AAT CGT AGC TGC GAG GTG CCA ACA

       Arg Leu Asn Ser Ala Ser Leu Lys Gln Pro Tyr Ile Thr Gln Asn Tyr Phe 119
505    AGG CTA AAT TCT GCA TCC CTC AAA CAG CCT TAT ATC ACT CAG AAT TAT TTT

       Pro Val Gly Thr Val Val Glu Tyr Glu Cys Arg Pro Gly Tyr Arg Arg Glu 136
556    CCA GTC GGT ACT GTT GTG GAA TAT GAG TGC CGT CCA GGT TAC AGA AGA GAA

       Pro Ser Leu Ser Pro Lys Leu Thr Cys Leu Gln Asn Leu Lys Trp Ser Thr 153
607    CCT TCT CTA TCA CCA AAA CTA ACT TGC CTT CAG AAT TTA AAA TGG TCC ACA

       Ala Val Glu Phe Cys Lys Lys Lys Ser Cys Pro Asn Pro Gly Glu Ile Arg 170
658    GCA GTC GAA TTT TGT AAA AAG AAA TCA TGC CCT AAT CCG GGA GAA ATA CGA

       Asn Gly Gln Ile Asp Val Pro Gly Gly Ile Leu Phe Gly Ala Thr Ile Ser 187
709    AAT GGT CAG ATT GAT GTA CCA GGT GGC ATA TTA TTT GGT GCA ACC ATC TCC

       Phe Ser Cys Asn Thr Gly Tyr Lys Leu Phe Gly Ser Thr Ser Ser Phe Cys 204
760    TTC TCA TGT AAC ACA GGG TAC AAA TTA TTT GGC TCG ACT TCT AGT TTT TGT
```

Fig. 11 (part 1 of 2)

```
        Leu Ile Ser Gly Ser Ser Val Gln Trp Ser Asp Pro Leu Pro Glu Cys Arg 221
811 CTT ATT TCA GGC AGC TCT GTC CAG TGG AGT GAC CCG TTG CCA GAG TGC AGA

        Glu Ile Tyr Cys Pro Ala Pro Pro Gln Ile Asp Asn Gly Ile Ile Gln Gly 238
862 GAA ATT TAT TGT CCA GCA CCA CCA CAA ATT GAC AAT GGA ATA ATT CAA GGG

        Glu Arg Asp His Tyr Gly Tyr Arg Gln Ser Val Thr Tyr Ala Cys Asn Lys 255
913 GAA CGT GAC CAT TAT GGA TAT AGA CAG TCT GTA ACG TAT GCA TGT AAT AAA

        Gly Phe Thr Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp 272
964 GGA TTC ACC ATG ATT GGA GAG CAC TCT ATT TAT TGT ACT GTG AAT AAT GAT

        Glu Gly Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Lys Ser Leu Thr 289
1015GAA GGA GAG TGG AGT GGC CCA CCA CCT GAA TGC AGA GGA AAA TCT CTA ACT

        Ser Lys Val Pro Pro Thr Val Gln Lys Pro Thr Thr Val Asn Val Pro Thr 306
1066TCC AAG GTC CCA CCA ACA GTT CAG AAA CCT ACC ACA GTA AAT GTT CCA ACT

        Thr Glu Val Ser Pro Thr Ser Gln Lys Thr Thr Thr Lys Thr Thr Thr Pro 323
1117ACA GAA GTC TCA CCA ACT TCT CAG AAA ACC ACC ACA AAA ACC ACC ACA CCA

        Asn Ala Gln Ala Thr Arg Ser Thr Pro Val Ser Arg Thr Thr Lys His Phe 340
1168AAT GCT CAA GCA ACA CGG AGT ACA CCT GTT TCC AGG ACA ACC AAG CAT TTT

        His Glu Thr Thr Pro Asn Lys Gly Ser Gly Thr Thr Ser Gly Thr Thr Arg 357
1219CAT GAA ACA ACC CCA AAT AAA GGA AGT GGA ACC ACT TCA GGT ACT ACC CGT

        Leu Leu Ser Gly His Thr Cys Phe Thr Leu Thr Gly Leu Leu Gly Thr Leu 374
1270CTT CTA TCT GGG CAC ACG TGT TTC ACG TTG ACA GGT TTG CTT GGG ACG CTA

        Val Thr Met Gly Leu Leu Thr Stop                                     381
1321GTA ACC ATG GGC TTG CTG ACT TAG CCAAAGAAGAGTTAAGAAGAAAATACACACAAGTA

1380TACAGACTGTTCCTAGTTTCTTAGACTTATCTGCATATTGGATAAAATAAATGCAATTGTGCTCTTC
1447ATTTAGGATGCTTTCATTGTCTTTAAGATGTGTTAGGAATGTCAACA
```

Fig. 11 (part 2 of 2)

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C12N15/12   C07K14/705   A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C12N   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	CARAS, I.W. ET AL.: "Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins." NATURE. vol. 325, 5 February 1987 (1987-02-05). pages 545-8. XP002109227 the whole document ---	20-25
A	DURRANT, L.G.: "Cancer vaccines." ANTI-CANCER DRUGS. vol. 8, no. 8, September 1997 (1997-09). pages 727-33. XP002109228 abstract ---	1-25
A	WO 97 32021 A (CANCER RES CAMPAIGN TECH ;SPENDLOVE IAN (GB); ROBINS RICHARD ADRIA) 4 September 1997 (1997-09-04) the whole document ---	

-/--

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1999	27/07/1999

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Authorized officer

Smalt, R

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	JUHL. H. ET AL.: "Frequent expression of complement resistance factors CD46, CD55, and CD59 on gastrointestinal cancer cells limits the therapeutic potential of monoclonal antibody 17-1A." JOURNAL OF SURGICAL ONCOLOGY, vol. 64, no. 3, March 1997 (1997-03), pages 222-30. XP002109229 abstract	
A	EP 0 685 739 A (TSUJI TAKAO ;SANKO JUNYAKU KK (JP)) 6 December 1995 (1995-12-06) the whole document	
P,X	SPENDLOVE. I. ET AL.: "Purification of 791Tgp72 antigen." IMMUNOLOGY, vol. 95. no. Suppl., December 1998 (1998-12). pages 32-Abstr.10.13. XP002109230 the whole document	1-25
P,X	WO 98 33523 A (BIOVATION LIMITED ;CARR FRANK JOSEPH (GB); CARTER GRAHAM (GB)) 6 August 1998 (1998-08-06) page 20. line 8 - line 18; claim 20	1-19
T	SPENDLOVE, I. ET AL.: "105AD7 anti-idiotype antibody is able to stimulate immune responses to and show similarity to a member of the DAF family." PROCEEDINGS OF TEH AMERICAN ASSOCIATION FOR CANCER RESEARCH. vol. 39, March 1998 (1998-03). pages 264-Abstr.1805. XP002109231 the whole document	1-26

1

Box I   Observations wher   certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority. namely:

   Remark: Although claim 19 is directed to a method of treatment of the
           human/animal body, the search has been carried out and based
           on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out. specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application. as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee. this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant. this International Search Report
   covers only those claims for which fees were paid. specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently. this International Search Report is
   restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

                               [ ] No protest accompanied the payment of additional search fees.

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